Acta

OTO-LARYNGOLOGICA

VOL. 64 JULY–DECEMBER 1967 FASC. 1–6

ACTA OTO-LARYNGOLOGICA L. 1 6. 19 NARVAVÄGEN 16, STOCKHOLM NO

Almqvist & Wiksells
BOKTRYCKERI AKTIEBOLAG
UPPSALA 1967

INDEX AUCTORUM

Vol 64

Almqvist & Wiksells
BOKTRYCKERI AKTIEBOLAG
UPPSALA 1967

INDEX AUCTORUM

Vol 64

INDEX ACTORUM

Vol 64

INDEX ACTORUM

Vol 64

PAUL FRENCKNER IN MEMORIAM

Professor Paul Frenckner Editor in-Chief of Acta Oto-Laryngologica, died on May 20th, 1967 During the past few years, he had been seriously ill for long periods, and had been hospitalized at intervals. A recent major gastric operation initially seemed to have been succes ful but complications ensued and led t his sudden death

Frenckner had dev ted himself to otolaryngology at an early tage Even during his first years at the Ear Nose and Throat Clinic of Karolinska Institutet, he showed outstanding technical aptitude which attracted the attention of his teacher Gunnar Holmgren. It was he who arranged f r Frenckner to visit Philadelphia in the early 1930 s, to study peroral endoscopy under Chevalier Jackson, the greatest laryng logist at the time Frenckner is said to have been imm nsely fascinated by Jackson who, in turn, soon singled out his Swedi h pupil

After his studies at Jackson s clinic, Frenckner became the pi neer of peroral endoscopy in Sweden He was not content merely to be the leading endoscopist from the clinical point of view It was not long before he started to design his own instruments, and to engage himself in fundamental research in this relatively new field

Early on, Frenckner established contact with our then foremost lung speciali t, Professor H. C. Jacobaeus, and his vari u co-workers, in particular Stig Björkman. The resulting intense collaboration led to construc tion f the double bronchoscope described in Frenckner's thesis for the doct rate in 1934 This instrument provided us with hitherto unsuspected possibilities f examining each lung separately Frenckner s position in the first rank as regards the diagnosi of lung disease led to close participation in the work of the lung surgeon Clarence Crafoo d and his vital contribu tion to the ad ances in lung surgery During that time I had the privilege —as the y ungest co-worker—of observing this important period of development, and I till retain a strong impres ion f Frenckner s passionate con tribution of labour and research. The aim was to improve the methods of diagnosis and research—n t least t improve and refine the anaesthetic procedures which, in the middle of the 1930 s, were still much neglected. Frenckner and Crafoord's teamwork was a prerequisite f r this development

Peroral endoscopy and its application in various fields were perhaps the essential features f Frenckner s research. Nevertheless, he had a lively nterest in all aspect f otolaryngology both clinical and theoretical. It would take too l ng to enumerat all hi practical, clinical contributions, but it can be mentioned that laryngology was fo a long time his favourite bject In addition to mastering the surgical techniques, he carried out distingui hed experimental research on the cilia over a period of years. Since Holmgren s research w s centered on otology particularly the surgical



Acta oto-laryngologica 64 5–16 (1967)

THE PERIPHERAL VASCULAR RESPONSE IN AUDIOMETRIC TESTING

B Drettner
Uppsala Sweden

From the Department of Otolaryngology (Head Prof A Sjöberg)
University of Uppsala

Variation in peripheral blood flow in response to auditory stimuli with pure tones were studied in 71 subjects. Adults and children with normal and impaired hearing and newborns were included A vasoconstrictive response was obtained in all adults and most children with normal hearing at mean and median threshold of 85–90 dB, respectively Adults and children with hearing loss revealed a vasoconstrictive response less often and at a higher median threshold. The thresholds, however showed such great dispersion as to greatly limit the practical value of this method in audiometric testing of adults and children The vasoconstrictive response occurred only infrequently in newborns.

The crucial importance of detecting hearing impairment before the onset of speech has led to the investigation of hearing tests relying on non verbal behavioral or autonomic responses. The present study seeks to evaluate the peripheral vascular response (PVR) in audiometric testing

Lehmann (1955) observed a vasoconstriction of the skin of the forehead during stimulation with white noise of 90 phon. Jansen *et al* (1964) recorded a vasoconstriction in the finger during exposition for white noise of 70 dB, but intensities less than 60 dB did not cause any reaction. Straneo, Galiot & Seghizzi (1962) found that noise of 80 dB had an angiospastic effect, measured by finger plethysmography in normal hearing subjects noise of 90–95 dB was required, when there were moderate defects of hearing, and no reaction was forthcoming in deaf mutes or patients with severe hearing loss.

Kollmeyer (1961) studied peripheral vascular reactions, using a modified plethysmograph placed on the fingertip Sound stimulation by means of floating tones ("Schwebungsklänge") proved more effective in eliciting vascular reactions than pure tones. 250 sound-stimulations administered to 20 adults elicited a peripheral vasoconstriction in 67% the mean threshold in this response exceeded the threshold of hearing by only about 10 dB In another study involving 32 subjects, the number of positive reactions using sound stimuli at the threshold of hearing was almost identical with that obtained using tones 10, 20 or 30 dB above threshold. Similar studies using the psychogalvanic skin response revealed positive results in only 40%

treatment of otosclerosis, Frenckner was naturally engaged in this work as well and he became a leading surgeon in the field I recall that Gunnar Holmgren once told me during one of the last years of his life, that he had seen most of the famous surgeons in the world perform the fenestration operation, but none with such an unparalleled technique as Frenckner This could be confirmed by many of his pupils Frenckner was one of the pioneers in the surgical treatment of Menière s disease, and also partook from the start in development of the tympanoplasty procedure

Frenckner served as a model for a whole generation of Swedish otologists, especially as an outstanding clinician Many crowded around his operating table, and we owe him a debt of gratitude for all his practical guidance.

When Gunnar Holmgren died in 1954 Frenckner was the obvious choice as Editor in-Chief of Acta Oto-Laryngologica, a post which he filled in an exemplary way from then onwards The leadership of this journal did, in fact become his most important task during the later part of his life We who were in close contact with him knew that his aim was to maintain the highest possible standard and thus to carry on the tradition of Holm gren These two—Holmgren and Frenckner—made contributions to Acta Oto-Laryngologica that will never be forgotten Their constant endeavour was to increase in this way scientific collaboration all over the world, as well as to keep Scandinavian research on the highest international level

Frenckner underwent his whole clinical training under Gunnar Holm gren first at the old University clinic at Sabbatsbergs Sjukhus, and later at Karolinska Sjukhuset For a long time, he was Holmgren s second-in command, his task being to manage the clinic in every respect not least to train future specialists. He became Head of the Ear Nose and Throat Clinic at Sodersjukhuset in Stockholm in 1946, and stayed there until he became Professor Emeritus in 1961 At this hospital as well he carried on research and teaching of the highest class. His private practice in Stockholm assumed large proportions In view of his skill and reputation, his patients came from all over the country His burden of work was sometimes unbearably heavy even if his capacity was far above the average

Paul Frenckner s death implies a tremendous loss for otolaryngology both in Sweden and abroad For Acta Oto-Laryngologica it is a great blow and the void left will be hard to fill I am nevertheless convinced that all those responsible for the journal will do their utmost to carry on his tradition I am also convinced that continuing to work for Acta Oto-Laryngologica in the spirit of Paul Frenckner would have been his most sincere wish

C A Hamberger

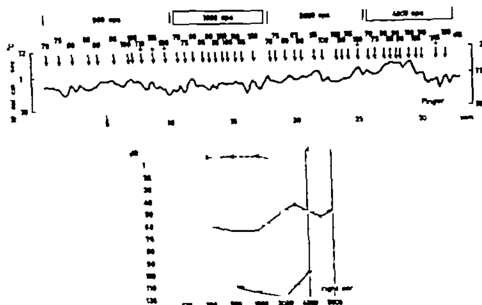


Fig. 2. Recording of the thermal conductivity of finger during sound stimulations to the right ear of child with congenital bilateral meatal atresia. Positive reaction with decrease of the thermal conductivity immediately after exposition for tone re marked with +. The threshold for PVR (▼——▼) is shown together with the audiogram at the bottom of the figure.

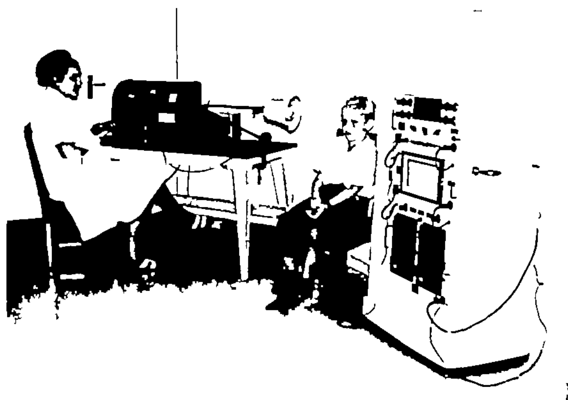
ment was adjustable in 5 dB steps in the range between 70 and 120 dB (re 0.0002 dynes/cm²). The maximal error on repeated calibrations was ±2 dB.

Each signal was about one second in duration. The sound levels were increased from 70 dB to 120 dB for each frequency. The length of the pauses between the signals was dependent on the vascular reaction, so that a signal was not given until the vascular reaction on the previous signal had completely subsided. An examination of both ears took from 1 to 2 hours.

The distance between the tested ear and the loudspeaker was 30 cm. The infants were positioned lying on their sides with the ear not being tested against the pillow. In older children and in adults glass-wool was used to shield the other ear.

Both adults and children were examined in a "silent" room. The neonates were tested in an ordinary room which was well sound insulated. Only the subject and the examiner were present during the examination.

Changes in the peripheral blood flow were studied by recordings of the thermal conductivity of the skin according to the method described by Hensel & Bende (1956). A commercially available apparatus (Fluvograph, Hartmann & Braun) was used. The measuring plate of the instrument was applied to the volar aspect of the terminal phalanx of the middle finger in adults and older children, while the heel was used in smaller children and infants.



F a. 1 The experimental equipm nt To th l ft th audiometer amplifi att nuat r and l udspeaker to the right th Flu ograph. Th photograph was taken in a different room than the and l olated one in which the examination were performed

MATERIAL

Altogether 71 subjects were examined 19 adults, 17 to 82 years old 33 children, 6 months to 11 years old and 19 neonates, 12 hours to 11 days old All of the adults and most of the children were patients at the Oto-Laryngological Department, University Hospital of Uppsala the remainder of the children were from a nursery school for children with severe hearing loss. The newborns were from the Department of Obstetrics. In all adults and most children hearing tests were performed with pure tone or play audiometry using an Amplivox model 61 audiometer calibrated according to BS 2497 1954 Speech audiometry and/or Békésy audiometry was also performed in selected cases. The auropalpebral reflex (APR) was used in evaluating reactions in neonates and small children

METHODS

The subjects were exposed to pure tones in the frequency range of 500 1000 2000 and 4000 cps The apparatus consisted of an audiometer with power amplifier and an attenuator connected to a loudspeaker on a m v able arm (Fig. 1) The instrument was calibrated by means of a sound level meter and a condenser microphone placed at right angles to the loudspeaker at a distance of 30 cm The sound level at this distance from the instru

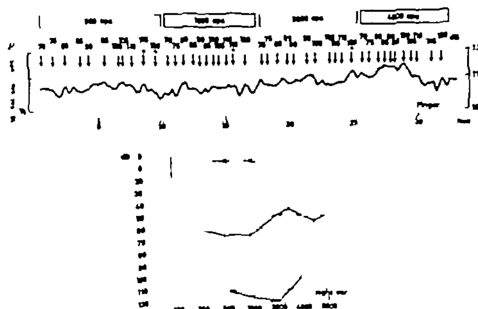


Fig. 2. Recordi g of the thermal conducti ity of fi ger duri g sound stim lations to the right ea of child with congenit l bilateral meat l tresia Positiv reaction with decrease of the thermal conducti ity immediately fter exposition for tone re marked with + The threshold f PVR (▼——▼) i show together with the audiogram in the bottom of the figure

ment was adjustable in 5 dB steps in the range between 70 and 120 dB (*re* 0.0002 dynes/cm) The maximal error on repeated calibrations was ±2 dB

Each signal was about one second in duration. The sound levels were in creased from 70 dB to 120 dB for each frequency The length of the pauses between the signals was dependent on the vascular reaction so that a signal was not given until the vascular reacti n on the previous signal had completely subsided. An examination of both ears took from 1 to 2 hours.

The distance between the tested ear and the loudspeaker was 30 cm. The infants were positioned lying on their ides with the ear not being tested against the pillow In older children and in adults glass-wool was used to shield the other ear

Both adults and children were examined in a "silent room The neonates wer tested in an ordinary room which was well sound-insulated Only the subject nd the examiner were present during the examination.

Changes in the peripheral blood flow were studied by recordings of the therm l conductivity f th skin according to th method described by H nsel & Bender (1936) A commercially a ailable apparatus (Flu ograph, H rtmann & Braun) was used The measuring plat of the instrument was pplied t the plantar aspect of the terminal phalanx of the middle finger in adults nd older children, whil the heel was used in smaller children and infants.

TABLE 1 *Results in adults*

Group	Number of subjects	Number of ears examined	Number of ears from which PVR was elicited cps 500	1000	2000	4000	Ranges of thresholds for PVR (in dB re 0.0002 dynes/cm²) cps 500	1000	2000	4000
Normal hearing	10	16	16	15	16	16	70–120	0–120	0–110	70–110
Conductive hearing loss	5	7	4	3	5	4	85–115	95–120	90–110	90–105
Sensorineural hearing loss	6	9	7	7	8	9	90–120	70–110	5–120	70–120

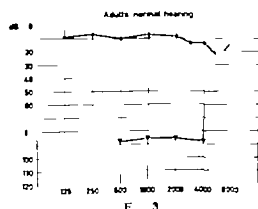
INTERPRETATION OF THE RECORDINGS

All unequivocal changes in peripheral blood flow upon sound stimuli were those of diminution i e a vasoconstriction Such a reaction was recorded by a steep decrease in thermal conductivity This was initiated within a few seconds after the sound stimuli (Fig 2) Spontaneous variations in the peripheral blood flow were sometimes recorded as small wave shaped variations, but these were generally less steep and less pronounced than those reactions caused by sound stimulation

RESULTS

Normal Hearing Adults

PVR was elicited in all normal hearing adults (Table 1) As seen in Fig 3 the mean threshold for eliciting PVR was 85–90 dB (*re* 0 0002 dynes/cm²) *The dispersion, however was considerable and occurred throughout*



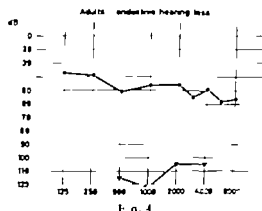


Fig. 3   Fig. 4

FIG. 3 M n thresh ld f th pure tone udiogr m nd mea thre h ld for PVR i n rmal h a i g d lt

FIG. 4 Mean thresh ld of th pure t udiogram d medi threshold f PVR in adult with co d t h ri g los

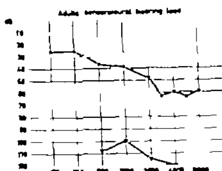


Fig. 5

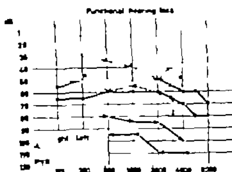


Fig. 6.

Fig. 5. Mean threshold of the pure tone audiogram and median threshold for PVR in adults with sensorineural hearing loss.
Fig. 6. Audiogram and thresholds for PVR in patient with functional hearing loss.

the whole tested range in the lower frequencies (Table 1). The difference between the mean threshold for the PVR and the mean threshold for the pure tone audiogram was 75–80 dB. However, different reference levels were used for the calibration of the Amplivox audiometer and the special audiometer for PVR-studies. Wedenberg (1963) who used similar reference levels, showed that the error caused by different reference levels did not surmount 5 dB in the range of 500–4000 cps so that no corrections for this variable are necessary.

Adults with Hearing Loss

Conductive hearing loss

Only from 3 of the 7 ears in this group could PVR be elicited in all tested frequencies. Total absence of PVR characterized the sound stimulation to two ears (Table 1). Since the arithmetical mean for the PVR thresholds could not be calculated in this group, the median of the threshold for PVR is presented (Fig. 4). The median threshold was higher at 500–1000 cps than at 2000–4000 cps.

Sensorineural hearing loss

Sensorineural hearing loss of cochlear type according to Békésy-audiogram was present in 9 ears (Menière's disease, presbyacusis, noise damage). PVR was elicited in all frequencies from 6 ears, and one ear gave a completely negative result.

Fig. 5 shows the median threshold for PVR in this group. This threshold was higher at 2000–4000 cps than at 500–1000 cps, but the dispersion was great (Table 1).

Functional hearing loss

One patient had a pronounced bilateral hearing loss according to pure tone audiogram, but answered adequately to questions in a low voice

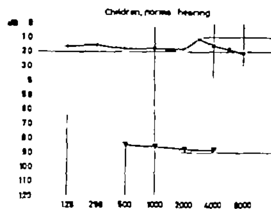


Fig 7

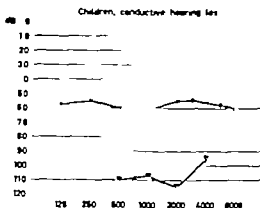


Fig 8

Fig 7 Normal hearing children: mean threshold of pure tone audiogram and median threshold for PVR

Fig. 8 Children with conductive hearing loss mean threshold of pure tone audiogram and median threshold for PVR

Simulation tests indicated a functional hearing loss. PVR was elicited in all tested frequencies from both ears (Fig 6) The threshold for PVR was somewhat higher in the right than in the left ear as was the threshold for the pure tone and Békésy audiogram A slight organic hearing loss, at least in the *right ear might therefore be present in addition to* her functional condition

Normal Hearing Children

In 10 children 2–11 years old 17 ears with normal pure tone audiogram were examined From 10 of these ears PVR was elicited in all frequencies, and from another in three frequencies One child could not keep quiet during the examination, which disturbed the recordings unequivocal PVR was recorded only at 4000 cps to each ear Absence of PVR characterized two children in whom both ears were examined One of these children had a cerebral palsy and the other had very cold and pale hands and feet during the examination The median threshold for PVR in the *normal hearing children (Fig 7) bears a close resemblance to the mean* threshold for PVR in normal hearing adults (see Fig 3) The ranges of the PVR thresholds are given in Table 2

Children with Hearing Loss

Conductive hearing loss

6 children 5–10 years, were examined The diagnoses were *congenital* meatal atresia (7 ears) and otosalpingitis (3 ears) PVR was elicited in all frequencies from 7 ears, and was absent from one The thresholds showed a great dispersion (Table 2) The median threshold for PVR and the mean threshold of the pure tone audiograms are given in Fig 8

TABLE 2 *Results in children*

Group	Number of subject	Number of ears examined	Number of ears from which PVR was elicited, cps 500	1000	2000	4000	Ranges of thresholds for PVR (in dB re 0.0002 dynes/cm²), cps 500	1000	2000	4000
Normal hearing	10	17	10	11	12	12	75–105	70–110	80–95	70–110
Conductive hearing loss	6	10	8	8	8	8	80–120	80–115	75–120	70–110
Sensorineural hearing loss	4	7	3	2	3	2	105–120	100–115	100–120	105
No audiogram normal ear drums	12	19	17	13	15	11	70–120	70–120	70–120	70–100

Sensorineural hearing loss

4 children, 2–6 years old, with bilateral severe hearing loss since birth were studied The cause of the hearing impairment was maternal rubella in the first trimester of pregnancy in two and unknown in the other two. Among the 7 ears tested 3 were characterized by total absence of PVR (Table 2) The number of positive reactions in this group was too few to allow any calculations of the median threshold for PVR

Children without Audiogram

In 15 children (/₂–4 years old) in whom no play audiogram was obtained, altogether 23 ears were tested. Normal ear drums were present in 19 ears the number of positive reactions among these and the ranges of the thresholds are presented in Table 2. One child showed neither PVR nor APR from any ear The median threshold for PVR in the 19 ears with normal ear drums is given in Fig. 9

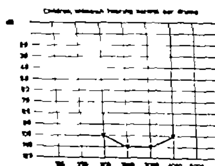


FIG. 9 Media threshold for PVR in those hildren i hom no diogram w obtained nd who had normal ear drums.

Pathological changes of the ear drums (acute otitis, otosalpingitis) were present in 4 ears from two of these PVR was elicited only in one of the frequencies and from the other two in none

Neonates

Studies of PVR and APR were performed in 19 neonates, 12 hours to 11 days old. Both ears were tested in all except one The newborns and their mothers were without illnesses likely to impair hearing The birth weight of these newborns was 2.290–3 890 g and body temperature at the time of the examination 35 8 C–37.2 C.

All neonates except one reacted with APR in all tested frequencies The exception was an infant in whom the APR test was completely negative on bilateral testing, but PVR was elicited from one ear by 1000 cps. The mean threshold for APR at 500 1000 2000 and 4000 cps among the 35 ears, from which APR was elicited, was 110 dB (ranges 95–120 dB) 111 dB (ranges 100–120 dB) 112 dB (ranges 100–120 dB) and 113 dB (ranges 100–120 dB) respectively These values are in fairly good agreement with those of Wedenberg (1963)

PVR was elicited only rarely in these neonates The number of positive reactions on this test was 5 at 500 cps and 2000 cps and 7 at 1000 cps and 4000 cps

Differences between the Thresholds for PVR and Hearing in Adults and Children

In those adults and children, showing PVR and in whom a hearing threshold could be determined the difference between the threshold for PVR and the threshold of the pure tone audiogram or play audiogram was calculated for each ear and each frequency Adults and children showed similar results, which were therefore combined (Table 3) The distribution of the results in the normal hearing subjects is not in close agreement with a distribution by random at the lower range which probably is explained by the fact that no tests were performed in intensities lower than 70 dB The distribution of the results in the other two groups is more consistent with a distribution by random

Comparison between the Occurrence of PVR and APR in Children (excl Newborns)

The occurrence of PVR and APR in different frequencies was compared in 16 children (26 ears) between the ages $^1/_2$–4 years. All these children are included in previous mentioned groups two children had normal play audiogram two others had bilateral sensorineural hearing loss due to maternal rubella and the rest were children in whom no audiogram was obtained The agreement between the presence and absence of PVR and APR was better at 500 cps than at the other frequencies (Table 4)

TABLE 3 *Distribution of the differences between the PVR threshold and the hearing threshold in ears from which PVR was elicited in at least one frequency*

		Difference in dB				
Subjects	cps	0–20	25–45	50–70	75–95	100–120
Normal-hearing (20 ears)	500			12	10	1
	1000			15	7	2
	2000			11	13	1
	4000		1	10	10	6
Total			1	48	40	10
Conductive hearing loss (17 ears)	500		5	7		
	1000	2	3	5	1	
	2000	1	2	8	2	
	4000	1	4	6	1	
Total		4	14	26	4	
Sensori-neural hearing loss (14 ears)	500	2	1	3	3	1
	1000	2	1	1	4	
	2000	1	4	2	2	1
	4000	1	3	2		1
Total		6	9	8	9	3

A complete absence of PVR in the presence of APR at some frequencies characterized 4 ears belonging to 3 children two of them having an organic brain damage. In one of these with symptoms of bilateral brain damage but normal play audiometry no PVR was elicited from any ear One child with a hemiplegia of the right side showed a good agreement between the thresholds for APR and PVR from the right ear and APR was elicited by 120 dB at 1000–4000 cps from the left ear but no PVR appeared. The third child had a bilateral hearing loss due t maternal rubella, but only one ear demonstrated a discrepancy in the eliciting of APR and PVR from the other ear both these tests gave negative results.

One two-year-old child showed PVR from one ear by 80–90 dB at all frequencies, but no APR appeared from this ear while both APR and PVR was elicited from the other ear His ear drums were normal and play audiometry one year later showed normal hearing on both ears.

TABLE 4 *Comparison between the occurren e of APR and PVR in children t 500–4000 cps (n = 26 ears)*

	500 cps		1000 cps		2000 cps		4000 cps	
	APR	No APR	APR	No APR	APR	No APR	APR	No APR
PVR	15	1	11	2	13	2	8	4
No PVR	4	6	[illegible]	6	7	4	7	7

TABLE 5 *Median threshold in dB (re 0 0002 dynes/cm²) for eliciting APR and PVR from 26 ears in children*

	500 cps	1000 cps	2000 cps	4000 cps
APR	115	120	115	120
PVR	113	120	115	>120

The median thresholds for APR and PVR among the 26 compared ears beared *a close resemblance at all frequencies* (Table 5) but the ranges were different The measurable APR thresholds varied between 105 dB and 120 dB in all tested frequencies, while the ranges of the corresponding PVR thresholds was 70–120 dB at 500 1000 and 2000 cps and 70–100 dB at 4000 cps A PVR threshold not differing more than ±10 dB from the APR threshold was found in 7 ears at 500 cps, in 6 ears at 1000 cps and 2000 cps, and only in 3 ears at 4000 cps

DISCUSSION

The presented method differs from that of Kottmeyer (1961) and the results are therefore not directly comparable The desire to be able to correlate the results whith those of Wedenberg (1956 1963) concerning APR in children was the reason why pure tones of high intensity were used instead of floating tones, in spite of Kottmeyer s observation that the latter were more effective than pure tones in eliciting PVR

The results of the present investigation show that PVR was elicited in all normal hearing adults. The mean threshold for PVR in this group was 85–90 dB, but the lowest intensity of sound used in the present investigation was 70 dB and another investigation using also lower intensities might therefore give a lower mean threshold PVR was elicited less often in adults with hearing loss than in those with normal hearing and the median threshold was higher The dispersion of the thresholds was however too great to give the presented method for PVR any practical value as auditory test in adults, except possibly as a complement to other methods in revealing functional hearing loss Kottmeyer (1961) managed to unveil two cases of functional hearing loss with his method

In children with normal hearing the median threshold for PVR was 85–90 dB but with a great dispersion Falsely negative tests with complete absence of PVR occurred Cold and pale hands or feet, indicating a peripheral vasoconstriction already before the sound stimulation appears to be one cause to falsely negative results, and another seems to be organic brain *damage* Children with hearing loss had as expected a higher median threshold for PVR than those with normal hearing but the dispersion was great also among these The practical value of PVR as an objective *auditory* test in children seems therefore to be small The investigation

confirms the observation of Wedenberg (1963) that the APR-test can be used with success also after the neonatal period. The ranges of the threshold for the latter reaction are much smaller than the ranges of the PVR thresholds. Examination with auditory elicited PVR may thus have a value only in those few children, in whom the usual hearing tests have been unsatisfactory and the APR-test has yielded a negative result, which is d ubted.

The occurrence of PVR on sound-stimuli in neonates was too infrequent to gi e this test any value in the examination of the hearing of newborns. The reason why neonates do not yield a PVR to sound more co mmonly is obscure Hearing has been hown to be present before birth (Johansson, Wedenberg & Westin, 1964) A vasomotor activity of kin is found in the neonatal period, at least on thermal stimuli (Brück, Brück & Lemtis, 1960 Young, 1962) and the heel i u ually the place where such reaction are especially pronounced in newborns (Brück, 1961) A body temperature between 36.5 and 37.5 C is con idered to be necessary for eliciting vascular reflexes in neonates (Brück, 1961) but most neonates in the present investigation had a body temperature within these ranges. The peripheral vasomotor activity on sound stimulation seem therefore to be much weaker in newborns than in adults and children.

This investigation confirms the results of Wedenberg (1956, 1963) that the dispersion f the thresholds for APR is mall in newborns, which is of great importance when using thi reaction as an objective auditory test.

ZUSAMMENFASSUNG

Variation im peripheren Blutfl ss als Reaktion a f auditorische Reize mit einen Tonen wurden bei 71 Personen untersucht. Der Versuch umfasste Erwachsene und Kinder mit n rmalem und erminderteni Hörvermögen und Neugeboren Eine gefässverengernd Antwort w rde bei allen Erwachsenen und den m len K dern mit ormalem Gehör erzi lt, d durchschnittl che bzw mediane Schw llenwert belief sich auf 85–90 dB. Bei Erwachsenen nd Kindern mit ver m derten Horvermogen kam die gefässverengernde Antwort weniger häufig vor und gl men höheren medianen Schwellenw rt auf D Schw llen zeigten jedoch solch e grosse Vert lung, da sie d n praktischen Wert dieser audiometrischen Testmethode be Erwa hse en und Kindern beträchtlich begrenzen. Die gefass erengernd Antwort trat nur selten ein bei Neugeborenen.

REFERENCES

Br x, K., 1961 Temperature regulation in the newborn inf nt *Biol N onat* 3 65.

Br ck, K., Brück, M., and Lemtis, H 1960 Di Temperaturreg lung Neugeborener nd Frühgeborener nach spontaner nd pathologischer Geburt *G burtsh Frau nh ilk* 2, 461

H nsl, H nd Brecht, F 1936 Fortlaufende Bestimmu g der Hautdurchblut ng am Menschen mit einem lektrischen Wärmel itmesser *Pflüge Arch Ges Physiol* 263, 603.

JANSEN G ROSCA, S SCHULZE, J PLETTER, D and EL MOFTY A 1964 Vegetative reactions to auditory stimuli *Trans Amer Acad Ophthal Otolaryng* 68 445

JOHANSSON B WEDENBERG, E., and WESTIN B., 1964 Measurem nts of tone re ponse by th human foetus. *Acta Ot laryng* (Stockh) 57 188

KOTTMEYER, G 1961: *Plethysmographische Untersuchungen nach schwellennahen sensorischen und sensiblen Reizen Arch Ohr Nas Kehlkopfheilk* 177 297

LEHMANN G 1955: *Untersuchungen ur Frage der n ösen Lärmbelastung Karl Arnold Festschrift* Westdeutscher Verlag Köln and Opladen

STRANEO, G GALIOTO, G B and SEGHIZZI, P 1962: La rispo ta pletismografica allo stimolo sonoro possibilità d lla sua applicazione n lla simulazione della sordità *Boll Mal Ore h* 80 101

WEDENBERG, E., 1956 Auditory tests on new born infants. *Acta Otolaryng* (Stockh) 46 446

— 1963 Objective auditory tests on non-cooperative children *Acta Otolaryng* (Stockh.) Suppl 175

YOUNG, I M 1962 Vasomot r ton in the skin blood vessel of the newborn inf nt *Clin. Sci* 22 325

Dept of Otolaryngology
University f Uppsala
Uppsala, Sweden

Received Oct ber 17 1966

\cta oto-laryngologica 64 17 29 (1967)

ELECTRON MICROSCOPIC AND HISTOCHEMICAL IDENTIFICATION OF LIPOFUSCIN IN THE HUMAN INNER EAR

T Ishii, Y Murakami R S. Kimura and K. Balogh, Jr
Boston Mass U.S A

From the D partment of Otolaryngology Massachusetts Eye and Ear Infirmary and th D partments f Pathology Massachusetts General Hospital and Harva d Medi al School

Normal inn ear f 14 indiv lual ranging from 2 day t 91 years of age were studied with electron m croscopi and histochemical techniques for th distribution of l pof scin and lysosomal enzymes (acid phospha- t se beta-glucuronidase a d N-acetyl-beta-glucosami idase) The electron m croscope revealed in the cochlea la ge membrane-limited inclusion in the ap cal cytoplasm f all ep th lial cell lining the ndolymphati pace Sim lar inclusion were also seen in the estibular pp ratu th pical zone f the sensory nd support ng lls, s well the tran tion l ep th l um nd dark cell contained numerou l pofuscin granules. I the sam location where these struct res occurred, w ha bserved trac toplasmic gran les w th the histochemical characteristics f l po- fusc n luding y llow to green ut fluorescence The sites of lipofuscin accum lation lso d played strong acid pho phat se acti ty Lipofuscin uld not be demonstrated histoch mical n the newbo rn d was nly sparse in ch ldren, but with ge t gr duall ncreased in qua tity On the tt ha d d phosph tase ctt t w practically the same in d idual f ll ages. Th ge-dependent increase f lipofuscin in the hum n inner ea app rentl soci ted w th lysosomal ctivity

INTRODUCTION

M croscopi t ha e bserv d a long time go that lipofuscin accumulates n the ti ues f old individuals, hence it popular syn nym "wear-and-tear pigment and its German name Al nutzungspigment" (Lubarsch, 1902 Sch rt, 1904 Hueck, 1912

Great progres w made in the last decad when cyt chemical and ultra tru tural in estigation establi hed the relationship between lipofuscin and lysosomes in v riety of ti ues f lifferent species. Thus, it wa ec gnized that certain ll rg nelles are involved in the f rmation of l pof iscin Although cellula rgan lles have been investigated in arious

This study was supported by rese rch gr nt NB 04133-03 nd NB 03032-03 from the N tional Instit t f Neurological D seases nd Blindness, N ti l Institutes of Health of the U ited St tes Public Health Service

tissues for morphological changes associated with aging, the inner ear has not been subjected to such a study Therefore we have examined with histochemical and electron microscopic techniques a series of human inner ears for the presence of lipofuscin and lysosomal enzymes The present communication reports the results of this research

MATERIAL AND METHODS

Normal temporal bones of 14 individuals ranging from 2 days to 91 years of age were examined None of these patients had a history of hearing disorder or middle ear disease

Electron Microscopy

Human cochleae were removed for electron microscopy as described elsewhere (Kimura Schuknecht & Sando 1964) Six specimens, from individuals with ages ranging from 54 to 86 years were fixed in 1% veronal buffered osmium tetroxide, cut with an LKB ultrotome stained with uranyl acetate and lead citrate examined and photographed under the Siemens Elmiskop I with magnifications ranging from ×1000 to 40 000 Thick sections were examined with a phase contrast microscope

Histochemistry

The temporal bones of 8 patients were removed 4 to 6 hours postmortem trimmed and fixed for 24 hours in a cold (4 C) 4% neutral formol-calcium solution Afterwards, the stato-acoustic nerve the cochleae ampullar cristae and macula utriculi were dissected under the otomicroscope (Ishii Murakami & Balogh 1967) Then the cochleae were decalcified for six hours in a cold (4 C) 10% EDTA solution in a 0 2 M phosphate buffer at pH 7 45 (Balogh 1962 and 1965) Thereafter the tissue blocks were frozen on Dry Ice mounted and cut at 12 μ in a cryostat (−25 C) The frozen sections were mounted on clean coverslips, gently thawed and briefly dried at room temperature *The sections were used for one of the following histochemical studies.*

Lysosomal enzymes

Acid phosphatase (Barka & Anderson 1962) β glucuronidase (Hayashi Nakajima & Fishman 1964) and N acetyl β glucosaminidase (Hayashi 1965) activities were demonstrated by using the various naphthol AS-BI salts of the corresponding substrates Hexazonium pararosanilin served as a simultaneous coupler Nuclei were stained with methyl green Nonspecific reactions were ruled out by incubating sections in media from which the substrates have been omitted

Fluorescence microscopy

Some sections were pretreated for 12 hours in chloroform methanol (2 1 vol) at 37 C (hoenig, 1962) while others were directly mounted in buffered glycerol and examined with a Zeiss fluorescence microscope An Osram superpressure mercury burner HBO 200 W served as a light source excitation filter Schott BG 12 and barrier filter No 53 were used Microphotographs were taken on a Kodak high speed Ektachrome film.

Other histochemical tests

The periodic acid-Schiff reaction was done before or after treatment with diastase. Lipofuscin was demonstrated with the Schmorl test (Adams, 19 6) Pe l's method for ferric I n (Bunting, 1949) and the oil red 0 stain for neutral fat (Lillie 1954) were also employed in some instances.

OBSERVATIONS

Electron Microscopy

The organ of Corti consistently demonstrated large granular inclusions in the apical cytopla m of the epithelial cells lining the endolymphatic space (Fig 1 a) These inclusions were often bounded by a single limiting mem brane and varied in shape and size (0.2 to 1.5 μ) They contained single or multiple large oval lipoid globules, numerous smaller electron dense gran ules nd filamentou structures (Fig. 1 b) In sensory cells they were most often found near the Golgi network and lysosomes, and were les frequent in the area adjacent t the nerve endings. They were seen in great numbers in the narrow part f long Deiters cells processes, and scattered in the reti cul r lamina Hensen s cells, Claudiu cells and the sulcus cells showed similar inclusions near thei free surface The pillar cells also contained these large inclusions in th region peripheral to the intracellular tonofib ils.

In the vestibular apparatus similar large inclusions were most frequently seen in the apical zone of the sensory and supporting cells (Fig. 2 a) The transitional pithelium located between the neuroepithelium and the dark ells of Iwata (1924) also showed large inclusions (Fig. 2 b) These struc tures w re sometimes a large as the diameter of the cell nucleus, i.e about 5 μ Vestibular ganglion cells also contained large intracytoplasmic inclusions.

Histoch mistry

The histochemical reactions w re n t significantly affected by post mortem changes. In unstained tissues, lipofuscin presented as a brown, granular or amorphous substance

Lysos mal n-ymes

Th sites f enzymatic activity were identified by amorphous or granular deposits of brilliant red azo dye in the cytoplasm f various cells.



F 1 Electro m crograph f t se sory cell from the cochl f 63- ea -old m Lip f se g l re ce trated i th pl l ext pl m l Lipof sel gra ul cont l rg h eo l ma ses, ba ded truct res l u sero m ll d se gra ules.



Fig. 2. Electron micrographs of the crista ampull ri showing lipofusci gra les l the apic l zo of sensory nd upporting cell ( ) nd transiti nal epitheli m (b). Not d fference in the morphological haracteristics f these inclusions i Fig 1 b. Compa ble res re seen i Figs. 3 b nd 5 b, demonstrati g i these locations ccum la tion of utofluorescent nd oil red O positi granules, respecti ly

There were no significant differences in the distribution and intensity of acid phosphatase activity between the inner ear of a 2-day-old infant and that of a 91 year-old man Acid phosphatase activity was marked in the subcuticular portion of the auditory hair cells, apical zone of pillar cells, in Deiters cells, Hensen s cells (Fig 3 a) inner and outer sulcus cells, stria vascularis and cells of Reissner s membrane Intense acid phosphatase activity was seen throughout the cytoplasm of the spiral and Scarpa s ganglion cells (Fig 3 c) The limbus and Claudius cells showed moderate dye deposits. In the vestibular labyrinth there was marked acid phosphatase activity in the supranuclear zone of the neuroepithelium and transitional epithelium of the crista amullaris (Fig 3 b) Similar enzyme activity was demonstrated in the sensory cells and the supporting cells of the macula utriculi The activity of β glucuronidase and N-acetyl β glucosaminidase was in general much weaker than that of acid phosphatase Nevertheless, moderate activity could be observed in the subcuticular portions of the auditory hair cells, and in the spiral and vestibular ganglion cells Reissner s membrane was the only other site of noteworthy enzyme activity

Fluorescence microscopy

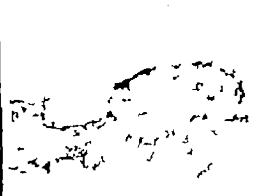
In the newborn, there were no fluorescent granules, but some were seen in the inner ear tissues of a 6-year-old girl In adults, strongly fluorescent yellow to green particles were found in the cytoplasm of various cells

Autofluorescence was not affected by a 12 hour lipid extraction with warm (37 C) chloroform methanol The fluorescent granules showed a consistent distribution pattern, although they tended to become more numerous and larger with increasing age Characteristically granules were limited to certain areas, such as the subcuticular portions of the auditory hair cells, the apical zone of Hensen s cells, Claudius cells and pillar cells (Fig 3 a) Reissner s membrane also contained many fluorescent particles, whereas the limbus, inner and outer sulcus cells, and the stria vascularis had only a moderate number The cells of the spiral ganglion also contained many fluorescent granules The apical portion of the vestibular sensory cells and supporting cells of the crista ampullaris and macula utriculi showed intense autofluorescence as did the transitional epithelium and the dark cells of the ampulla (Fig 3 b) Fluorescent granules were also distributed in the neural tissue of the ampulla and the utricular macula and were especially abundant in the cells of Scarpa s ganglion (Fig 3 c)

Other histochemical tests

The pigment granules gave a positive PAS-reaction that was resistant to previous digestion with diastase The pigment also stained with Schmorl s test for lipofuscin but did not contain demonstrable amounts of iron Lipofuscin is known to stain for lipid material stained with oil red O showed the same distribution pattern in the inner ear as the autofluorescent













Г 1



FIG. 4 Six-year-old girl. Oil red O tains lipofusci nd other lipids (black this micrograph) Compare with Fig 5 a. I the organ f Corti, small gra les re seen in the pper part of the inner and outer hai cells, Hensen cells, Deiters cells, Cl dius cells and pilla cells. Arrow points t tunnel f Corti 220 b. Only few granules re stained the crist mpullaris. 95.

FIG. 5. Eighty-one-year-old women. Sections st ned with oil red O. Org of Corti th abundant granules i the pical zones f I ner nd outer hai cells, Cl udius cells nd pilla cells. Arrow point t t nnel f Corti. Compare with Fig 3 a. 210. b. Crista ampullaris with many large granules i the neuroepithell m, transitional epithelium and ampullar nerve Compare with Fig 3 b. 140

granules (Fig 4) In old people, these granules were larger and stained intensely with oil red O especially in the subcuticular portion of the hair cells, in the apical zones of Hensen s cells and pillar cells, and in Claudius and Deiters cell (Fig 5 a) Correspondingly many large granules were also visibl in the vestibular labyrinth (Fig. 5 b)

Fig. 6 demonstrates the age-dependent accumulation of lipofuscin in the cells of Scarpa s ganglion There was no lipofuscin in a 2-day-old newborn (Fig. 6 a) whereas occasional pigment granules were present in some gangli n cell in a 6-year-old child (Fig. 6 b) and there was a great abundance of lipofuscin in the ganglion cells of an 84-year-old woman (Fig 6 c On the other hand, acid phosphatase showed a similar distribu-ti n pattern in all age groups (Figs. 6 a b c)

DISCUSSION

The biochemical and morphological concept f lysosomes was established by De Duve *et al* in 1955 Since th n many investigators have provided evidence that lysosomes participate in a number of m mmalian cell func tions, mong others in the intracellular digestion of endogenous and foreign

material Lysosomes are present in a great variety of tissues and are also involved in phenomena related to cellular injuries, tissue regression and necrosis. Characteristically lysosomes are rich in acid hydrolases at present at least 16 different enzymes have been identified in them (De Duve 1963) The morphological identity of lysosomes has been well established by electron microscopic studies of subcellular fractions of tissue homogenates (Essner & Novikoff 1960 Baudhuin & Beaufay 1963) by histochemical demonstration of acid phosphatase activity in tissue sections under the light microscope (Holt 1959 Novikoff 1959 1960 and 1963 Barka & Anderson, 1962) and with the electron microscope (Bondareff 1957 Essner & Novikoff 1961 Holt & Hicks, 1961 Samorajski Ordy & Keefe, 1965 Fawcett 1966)

In the inner ear of animals, lysosomes or lysosome-like granules were described by several electron microscopists (Rosenbluth, 1962 Rhodin, 1963 Bairati & Iurato, 1964 Spoendlin Schuknecht & Graybiel 1964 Hinojosa & Rodriguez Echandia 1966) Among others, lipofuscin granules were described in the stria vascularis of the guinea pig (Rodriguez Echandia & Burgos, 1965) These inclusions showed considerable morphological variations in different epithelia and even in the same cell type Such variants may be due to the heterogeneity of waste products or may represent different developmental stages of lipofuscin from lysosomes (Keefe & Ordy 1964 Strehler 1964 Samorajski Ordy & Keefe 1965) Bennett (1956) was the first to suggest that insoluble end products of metabolism (lipofuscin) may accumulate in lysosomes. Supporting this hypothesis is the fact that hydrolytic enzymes can be demonstrated histochemically and measured quantitatively in lipofuscin (Gedigk & Bontke 1956 Strehler *et al.*, 1959) Subsequent work (Essner & Novikoff 1960 Gordon Miller & Bensch, 1965) has confirmed Bennett's hypothesis The distribution of lipofuscin and lysosomal enzyme activity in the human inner ear also corresponds to the location of numerous large inclusions observed with the electron microscope in the human organ of Corti (Kimura Schuknecht & Sando, 1964)

Lipofuscin is a golden brown granular pigment that accumulates with age in the cytoplasm of various cells Nerve cells, myocardium and liver cells of older individuals are particularly rich in lipofuscin Its strong autofluorescence has been noted a long time ago (Bommer 1929 Hamperl 1934) The salient biochemical and histochemical features of lipofuscin were characterized by several investigators (Sulkin 1955 D Angelo Issidorides & Shanklin, 1956 Siebert *et al* 1962 Mytilineou Issidorides & Shanklin, 1963 Koenig 1963 and 1964) The pigment granules in the inner ear also have the histochemical characteristics of lipofuscin they stain with oil red O are positive with Schmorl's lipofuscin stain give a negative reaction for iron and remain PAS-positive after digestion with diastase Further evidence for the lipofuscin nature of these granules in the inner ear is their striking autofluorescence that is resistant to lipid solvents

In view of the relationship between lysosomes and lipofuscin it is f



FIG. 6 Saccular ganglion cells from 2-day-old newborn (nd) 6-year-old child (b nd b nd 84-year-old woman (nd c′) Oil red O stained sections (a, b and) on the left side are comparable with sections on the right demonstrating acid phosphatase activity (a′ b′ and c′) In the newborn, there is no lipofuscin ( ) whereas acid phosphatase activity can readily be demonstrated (a′) Some lipofuscin is present in the 6-year-old child (b) and many lipofuscin granules are seen in the 84-year-old woman. In contrast, acid phosphatase activity (a′ b′ and c′) is practically the same in individuals of all ages. 340.

considerable interest that lipofuscin was found in the human inner ear only at sites of acid phosphatase activity. This observation is in agreement with the results of histochemical and biochemical studies that have demonstrated the presence of acid hydrolases in cardiac lipofuscin (Gedigk & Bontke, 1956; Strehler *et al.*, 1959). It is also worth noting that the sites of lysosomal enzyme activity in the inner ear of man were essentially the same as in the guinea pig (Ishii & Balogh, 1966).

It is widely accepted that lipofuscin is an age-dependent pigment. In the inner ear it was not demonstrable in children under six years, but with age it gradually increased in quantity. This observation is approximately comparable with that of Strehler *et al.* (1959) who did not find lipofuscin in the myocardium of children under 10 years; however, in adults, quantitative methods revealed a clear linear increase with age.

While our studies do not permit statistically significant quantitation, it has been our impression that the amount of lipofuscin in the inner ear increases with age, i.e., the sensory epithelial and ganglion cells will show evidence of aging. Although the physiological significance of this phenomenon remains open for speculation, there are audiometric data (Rosée, 1953) that suggest decreased cellular function in the auditory neuron of old individuals. Analogously, there is a decreased vestibular response to mechanical and caloric stimulation in healthy aged people (Allard, 1938; Okano, 1938; Arslan, 1957). Whatever are the specific roles of lysosomes and lipofuscin in the human inner ear, at least one probable lysosomal function is the accumulation of endogenous or extracellular waste products. It can be concluded that age-dependent increase of lipofuscin in the inner ear apparently is associated with lysosomal activity.

ZUSAMMENFASSUNG

Im normalen Innenohr von 14 Menschen im Alter von 2 Tagen bis 91 Jahren wurde das Verteilungsmuster von Lipofuszin und lysosomalen Enzymen elektronenoptisch und histochemisch untersucht. Das Elektronenmikroskop zeigte im Cortischen Organ grosse, membran-umhüllte Einschlüsse im apikalen Zytoplasma aller Epithelzellen, die den endolymphatischen Raum säumen. Ähnliche Einschlüsse wurden auch im Vestibularapparat vorgefunden. Die apikale Zone der Sinnesepithelzellen und der Stützzellen, ferner die Übergangsepithelzellen und die sogenannten dunklen Zellen enthielten zahlreiche Lipofuszinkörnchen. In denselben Bezirken, wo diese Strukturen vorkommen, haben wir intracytoplasmatische Granula beobachtet, die die histochemischen Eigenschaften von Lipofuszin besitzen und demgemäss eine starke, gelblich-grüne Eigenfluoreszenz zeigen. Die Orte, an denen sich Lipofuszin regelmässig anhäufte, wiesen auch eine starke saure Phosphataseaktivität auf. Lipofuszin war im Innenohr von Neugeborenen histochemisch nicht nachzuweisen und auch im Kindesalter spärlich, jedoch nahm es mit dem Alter stufenmässig zu. Hingegen war die Aktivität der sauren Phosphatase praktisch unverändert in Individuen jeden Alters. Die altersabhängige Zunahme von Lipofuszin im menschlichen Innenohr scheint mit der lysosomalen Tätigkeit im Zusammenhang zu stehen.

REFERENCES

A AMS, C. W M., 1956 A stricter I terpret tion of the ferric ferricyanide reaction with particula reference t the demo stration f protein-bound lphydryl and di-sulphid groups. *J Histochem. Cytochem* 4 23.

ALLARD, A 1938 Contribution à l étud de la sensibilité de l' pparell semi-circulaire, pa la méthode des petites stimulations primaires, post-rotatoires, réalisées moyen du f uteuil Bays-Rylant. *A Otol 1* 417

ARSLAN, M 1957 The senescence of the estibular pparat s. *Pract Ot rhinolaryng* (Basel) *19* 475

BAIRATI, A nd I RATO S., 1964 Polarisationsmikroskopie, Röntgendiffraktion, Elek tronenmikroskopi I *Biochemie des Hörorgans* Ed. Rauch, S., Georg Thi me Verlag, Stuttgart, p. 406.

BALOGH, K., JR., 1962 Decalcification with Versene for histochemical st dy of oxidativ enzyme sy tems. *J Histochem Cytoch m.*, *10* 232.

— 1965 Histochemical localization of acid phosphatase activity i teeth after decalcification with EDTA. *J Histochem Cytochem* *13* 503.

BARKA, T nd A DERSON, P J 1962 Histochemical method for acid phosphatase sing hexazonium pararosanilin coupler *J Hi tochem Cytochem* *10* 741

BA CTN, P and BEA T, H., 1963 Examen microscope électronique de fraction purifiées d'orga tes cytoplasmiques de foie de rat. *Arch. I t. Physiol.*, *71* 119

BEVERIT H. S., 1956 A suggestion t the nature of the lysosome *J Bi phy Biochem Cytol* 2, Suppl., 185

BO MER, S., 1929 W itere Untersuchungen über ichtbare Fluoreszenz beim Menschen. *Act Dermatovene* (Stockh.) *10* 391

BONDAREFF W 1957 Genesis of I tracell lar pigment i the spinal ganglia of senile rats. A lectro microscope study *J Geront 12* 364

BU TING, H 1949 The histochemical detection of iron i tissues. *St in Tech* 24 109

D'ANGELO, C., ISSIDORIDES, M., and SHA KLIN, W 1956 A comparati tudy of the staining reactions f granules in the huma neuron. *J Comp Neurol 106* 487

DE DU E, C., 1963 The lysosome concept In *Lysosomes*. Ciba Foundation symposium. Ed. de Reuk, A. V S. and Cameron, M P Little, Brown & Co., Boston, Mass., p. 1

DE DUVE, C., PRESSMAN, B. C., GIA ETTO, R., W TTIAUX, R., nd APPELMANS, F 1955: Tissue fractionation studies. 6. Intracellular distribution patterns of enzymes in rat liver tissue *Biochem J 60* 604

ESS NS, E., nd NOVIKOFF A. B., 1960 H man hepatocellular pigment and lysosomes. *J Ultrastruct Res.*, 2, 374

— 1961 Localization of acid phosphatase activity in hepatic lysosomes by means of lectron microscopy *J Bi phys. Biochem Cytol* 9 773

F WCETT D W 1966 A *Atlas of Fine Structure Th Cell It Organ lles and Incl sions* W B. Saunders Co., Philadelphia, p. 281

GEDIGK, P and BONTKE, E., 1956 Über den Nachwei on hydrolytischen Enzymen in Lipopigmenten. *Z Zellforsch.*, 44 495.

GORDON, G B., MILLER, L. R nd BENSCH, K. G., 1965 Studies on the intracell lar digesti process in mammalian tissue culture cell *J Cell Biol* 25, 41

HAMPERL, H 1934 Die Fluoreszenzmikroskopie menschlicher Gew be. *Virchow Arch. Path Anat* 292, 1

H ASHI, M., 1965 Histochemical demonstration of N-acetyl β-glucosaminidase employ ing phthol AS-BI N-acetyl β-glucosaminide as substrat *J Histochem Cytochem.*, *13* 355.

H ASHI, M., NAKAJIMA, Y nd FISHMAN, W H., 1964 The cytologic demonstration of β-glucuronidase employing naphtol AS-BI glucuronid nd hexazo ium pararosanilin preliminary report *J Histochem. Cytochem* 12, 293.

considerable interest that lipofuscin was found in the human inner ear only at sites of acid phosphatase activity. This observation is in agreement with the results of histochemical and biochemical studies that have demonstrated the presence of acid hydrolases in cardiac lipofuscin (Cedigk & Bontke 1956, Strehler *et al.*, 1959). It is also worth noting that the sites of lysosomal enzyme activity in the inner ear of man were essentially the same as in the guinea pig (Ishii & Balogh 1966).

It is widely accepted that lipofuscin is an age-dependent pigment. In the inner ear it was not demonstrable in children under six years, but with age it gradually increased in quantity. This observation is approximately comparable with that of Strehler *et al.* (1959) who did not find lipofuscin in the myocardium of children under 10 years; however, in adults, quantitative methods revealed a clear linear increase with age.

While our studies do not permit statistically significant quantitation, it has been our impression that the amount of lipofuscin in the inner ear increases with age, i.e., the sensory epithelial and ganglion cells will show evidence of aging. Although the physiological significance of this phenomenon remains open for speculation, there are audiometric data (Rosée 1953) that suggest decreased cellular function in the auditory neuron of old individuals. Analogously, there is a decreased vestibular response to mechanical and caloric stimulation in healthy aged people (Allard 1938, Okano, 1938, Arslan, 1957). Whatever are the specific roles of lysosomes and lipofuscin in the human inner ear, at least one probable lysosomal function is the accumulation of endogenous or extracellular waste products. It can be concluded that age-dependent increase of lipofuscin in the inner ear apparently is associated with lysosomal activity.

ZUSAMMENFASSUNG

Im normalen Innenohr von 14 Menschen im Alter von 2 Tagen bis 91 Jahren wurde das Verteilungsmuster von Lipofuszin und lysosomalen Enzymen elektronenoptisch und histochemisch untersucht. Das Elektronenmikroskop zeigte im Cortischen Organ grosse, membran-umhüllte Einschlüsse im apikalen Zytoplasma aller Epithelzellen, die den endolymphatischen Raum säumen. Ähnliche Einschlüsse wurden auch im Vestibularapparat vorgefunden. Die apikale Zone der Sinnesepithelzellen und der Stützzellen, ferner die Übergangsepithelzellen und die sogenannten dunklen Zellen enthielten zahlreiche Lipofuszinkörnchen. In denselben Bezirken, wo diese Strukturen vorkommen, haben wir intracytoplasmatische Granula beobachtet, die die histochemischen Eigenschaften von Lipofuszin besitzen und demgemäss eine starke, gelblich-grüne Eigenfluoreszenz zeigen. Die Orte, an denen sich Lipofuszin regelmässig anhäufte, wiesen auch eine starke saure Phosphataseaktivität auf. Lipofuszin war im Innenohr von Neugeborenen histochemisch nicht nachzuweisen und auch im Kindesalter spärlich, jedoch nahm es mit dem Alter stufenmässig zu. Hingegen war die Aktivität der sauren Phosphatase praktisch unverändert in Individuen jeden Alters. Die altersabhängige Zunahme von Lipofuszin im menschlichen Innenohr scheint mit der lysosomalen Tätigkeit im Zusammenhang zu stehen.

1962 Isolierung und Eigenschaften on Lipof scl us Herzgew be des Menschen. *Histochemi* 3 17
SPOENDLIN, H H Sch ECHT H. F and GRAYBIEL, A 1964 The ultrastruct re of the ot lith organs in squirrel monkey fter expos re t high lev l of gra itoinertial force. *U S Naval Sch. Aviat Med Research R po t No. 102*
STREHLER, B. L., 1964 On the histochemistry nd ltrastruct re f g pigment. I *Advances i G tological Research Vol I* Ed. Strehler B L., Academic Press, New York nd London, p 313
STREHLER, B. L., MARK, D D MILDV A. S., nd GEE, M V 1959 Rat nd magnitude f ge p gment accum l tion i the h ma myocardium. *J G t 14* 430
S rt, N M 1955 Properties nd d stributio of PAS positi bst nces i the ervou stem f the senile dog *J G ront 10* 135

T I hii M.D Dept f Otolaryngology
Fac lty of Medicine U iversity f
Toky T kyo, J pan

Received November 21 1966

HINOJOSA, R and RODRIQUEZ ECHANDIA E. L., 1966: Th fine structure of the tria v s- nlaris f the cat inner ear *Amer J Anat 118* 631

HOLT S J 19 9: Factors gov rning the v lidity of staining method for nzymes, a d their bea ing upon the Gom ri cid phosphatase technique *Exper Cell Res.*, Suppl 7 1

HOLT S. J a d HICKS, R M 1961: The localization of acid phosphatase i rat liver cells as rev aled by combi ed cytochemical taining and lectron microscopy *J Bi phys Bioch m Cytol 11* 47

HULCK, W *1912: Pigmentstudien B itr path Anat 51* 65

ISHII, T and BALOGH, K., JR., 1966 Acid phosphatase tivity in the inner ear *Acta Otolaryng* (Stockh.) 6* 18.

ISHII, T MURAKAMI, Y nd BALOGH K JR., 1967: Acetylcholinest rase activity in the efferent nerve fibers f th human inner ea *Ann Otol 76* 69

IWAT N 1924 Ube da Labyrinth d Fled rmaus mit besonderer Berücksichtigung des tatischen Apparates. *Aichi J Exp Med* (Japan) *1* 42

KEEFE, J R nd ORDY J M 1964: Intracellular localization of lipofuscin age pigm nts In th nervou sy t m *J Ger nt 19* 262

KIMURA R S SCHUK KECHT H F and S ADO, I 1964 Fin m rphology f the sensory cell in the rg n f Corti of man *Acta Ot laryng* (Stockh) 58 390

KOC IG, H 1962 Hi tological distribution of brain gangliosides: Lysosomes as gly colipoprot in granules *Natu 19* 782

— 1963 Th utoflu rescenc f lysosomes It v lu f the id ntification of lys somal c tituent *J Histoch m Cyt ch m 11* 556

— *1964 N u al lipofuscin in disease its relati n to lysosomes. Trans. Am Neurol Ass 89* 212

LILLIE R D 1954 *Histopathologi T chnic and Pra tical Histochemi try* Th Bl kist n Di isi n, McGraw Hill Book Co New York, p 248

LUBARSCH O 1902: Über f tthaltig Pigm nt *Zbl Allg Path 13* 881

MYTILI EOU C, ISSIDORIDES, M a l SH K I W M 1963: Hi toch mi l reacti s of human t nomic g ngli *J An t 97* 533

NOVIKOFF A B 19 0 Lysosomes nd th physiology and pathology of cells. *B l Bull 117* 385

— 1960: Bioch mical and taini g re ti n of t pl mi co st tu nt In *D vel ping Cell Sy t m and Their Cont l* Ed Rudnl k, D Th R ld Press Co New York, p. 167

— 1963 Lysosom i the phy i log nd path l gy f cell Co trib tion of taini g method In *Ly os m s* Ciba F und ti ymp i m Ed de R u k, A V S and Cam ron, M P Littl Brown & Co Boston, Ma p 36

OKA O, H 1938 Klinisch st ti tisch Unters hu g n d J panisch n Greise In d m to-rhi o-l ryng logisch n Gebi te *J Ot hinolaryng Soc Jap 44* 1

RHO IN, J A G 1963: *An Atlas f Ultrastru tu* W B S d rs Co Phil d lphi nd Lond n, p 153

ROD GUE ECH NDIA, E. L., and BURGOS, M H 1965 The fine tru ture f the stri scul ri f th gui pig inn r *Z Z llf rs h 67* 600

ROSILE, B G 1953 U ters h g n über da H rv rmög n in d n verschied n Le-ben alt rn unter besond re Berück htigung d r Prüfu g mit d m Audi met r *Z Laryng Rhi ol Ot l* 3., 414

ROSENBLUTH, J 1962 The fi truct re f acoustic g ngl i th rat *J Cell B l 12* 329

S MORAJS L, T O Y J M and KEEFE, J R 1965 The fine structure f l pof scin age pigm nts the n rv y t m f ged mice *J Cell Bi l 26* 779

SEHRT E., 1904 Zu K nntnis d fetthaltig Pigm t V h w *Arch Path An t 177* 248.

SIEBER G DIEZEL, P B., JAHR, K KRUG, E., SCHMITT A GRÜNER OER, E., nd BOTTKE, I

TABLE 1 *Patients with hypopharyngeal pouch operated on during the years 1951–60*

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1 [illegible]	[illegible] ♂	3 years [illegible]				[illegible]
[illegible]	[illegible] ♀	1/2 year [illegible]				[illegible]
[illegible]	[illegible] ♂	1/2 year [illegible]				[illegible]
[illegible]	[illegible] ♂	1 1/2 years [illegible]				[illegible]
5 [illegible]	[illegible] ♀	4 years [illegible]				[illegible]
[illegible]	[illegible] ♂	2 year [illegible]				[illegible]
[illegible]	[illegible] ♂	1 year [illegible]				moderate
	[illegible]	[illegible] years [illegible]				[illegible]
[illegible]	[illegible] ♂	15 year [illegible]				[illegible]
[illegible]	[illegible] ♀	5 years [illegible]				[illegible]
[illegible]	[illegible] ♂	3 years [illegible]				[illegible]

muscular layer There was no death in the immediate postoperative period. Tw patients, however had complications in thi period. One of them had a pulmonary embolu and the other developed laryngeal edema which made it necessary t do a tracheotomy In no case was there mediastiniti r fi tula. B th patients wh were operated n twice had at the first opera tion ery mall pouches. The size of the diverticulas varied considerably the smallest being le than 1 cm and the largest being m re than 10 cm in diameter

Follow-up of Operated Patients

Six of the 31 operated pati nt ha e died of other diseases. All the other patient have been interviewed about their symptoms since the operation Tw lve patients are without any complaints. Nine patients remarked that

Acta oto-laryngologica 64 30–36 (1967)

ON THE TREATMENT OF ESOPHAGEAL DIVERTICULA

S Einarsson and O Hallen
Goteborg Sweden

From the Department of Otolaryngology (Head Prof G Herberts) Sahlgrenska Sjukhuset University of Gothenburg Göteborg

A follow up study has been made on 31 patients with hypopharyngeal pouch operated during the years 1951–60 and on 8 patients from the same period which have not been operated The patients have been interviewed about their symptoms and X-rayed Of the operated group six have died of other diseases, twelve are without any complaints, nine have mild and four more pronounced symptoms One patient concludes that the operation has not made her better The X ray findings were not exactly correlated to the symptoms of the patients. In the non-operated group the roentgenograms did not show any increase in size of the smallest diverticulas during a ten year period It is concluded that the etiology of hypopharyngeal pouches is unclear that cases in stage I should be treated conservatively cases in stage II or III operated by a one stage lateral pharyngotomy with excision of the pouch with or without division of the cricopharyngeal muscle

INTRODUCTION

In spite of the many articles written about esophageal diverticula during the last decades controversy still exists regarding both the etiology and treatment of this disease The purpose of this article is to report the material from a ten year period in our department and to discuss some of the problems.

MATERIAL

During the years 1951–60 31 patients with esophageal diverticula were operated on Two patients were operated on twice because of recurrences one and three years after the first operation During the same time period 8 patients with this disease were for various reasons, treated conservatively In the operated material there were 20 males and 11 females. The average age at operation was around 61 years The distribution in age groups was as follows 30–39 years, 1 patient 40–49 2 patients 50–59 8 patients 60–69 15 patients 70–79 3 patients 80–89 2 patients.

METHOD

The method used for all the patients was a one-stage operation consisting of lateral pharyngotomy resection of the diverticula and closure of the

TABLE 3 *Patients with hypopharyngeal pouch which for different reasons has not been operated on*

Case No. Age Years	Sex	Symptoms	[illegible]	[illegible]	1965 Symptoms
1 64	♂	1/2 year [illegible]	[illegible]	[illegible]	[illegible]
2 [illegible]	♀	1/2 year [illegible]	[illegible]	[illegible]	[illegible]
3 64	♀	1/2 year [illegible]	[illegible]	[illegible]	moderate
[illegible]	♂	1/2 year [illegible]	1957	[illegible]	[illegible]
5 65	♀	1 year [illegible]	1957	[illegible]	[illegible]
75	♂	[illegible] years [illegible]	[illegible]	[illegible]	[illegible]
7 [illegible]	♂	1/2 year [illegible]	[illegible]	[illegible]	[illegible]
8 [illegible]	♀	1/2 year [illegible]	[illegible]	[illegible]	[illegible]

cula were of a size smaller than 1 cm in diameter The roentgenograms taken 8, 9 and 10 years later show no increase in the size of the diverticula, and the patients report that symptoms have not changed (cases nos. 3, 4 and 8) Case no 6 was followed during a ten year period In spite of in reasing sympt ms nd repeated recommendations he did not accept operation Finall he came into the hospital in such a bad state that it was impossible to save him. The other four cases in this group ha e been fol lowed up to ten years. The symptoms as well as the size of the diverticulas ha e lowly increased.

DISCUSSION

Like most of the published f llow up investigations of esophageal diverticula this series is too small to allow any definite conclusions about the therapy of this disease However the relatively large numbe f operated patients who are not completely symtom free has inspired us to see if it is possible to get from the literature on this subject definite opini ns about the etiology and therapy of esophageal diverticula.

Etiology

Intraluminal pressure registration in the hypopharynx (Fyke & Code 1955 Atkinson *et al* 1957) how that the cricopharyngeal muscle re-

Table 2 *Patients with hypopharyngeal pouch operated on during the years 1951–60*

[illegible]	[illegible]	Symptom	Preop. [illegible]	[illegible]	1963 [illegible]	1963 Symptoms
11 / 74	[illegible] / ♂	2 [illegible]				1 [illegible]
12 / 75	[illegible] / ♀	10 [illegible]				[illegible]
13 / 63	[illegible] / ♂	2 [illegible]				[illegible]
14 / 71	[illegible] / ♂	1 1/2 [illegible]				
15 / [illegible]	[illegible] / ♀	1 1/2 [illegible]				[illegible]
16 / [illegible]	[illegible] / ♂	1/2 [illegible]				1 [illegible]
17 / [illegible]	[illegible] / ♂	1 1/2 [illegible]				1 [illegible]
[illegible] / [illegible]	[illegible] / ♀	[illegible]				[illegible]
19 / [illegible]	[illegible] / ♀	10 [illegible]				[illegible]
[illegible] / [illegible]	[illegible] / ♀	5 [illegible]				[illegible]

they have mild symptoms of the same kind as before operation and four patients have *moderate* symptoms Only one of the patients (case no 2) concludes that operation has not made her better

X ray controls

Of the 25 living operated patients it has been possible to X ray 20 In only three of the cases are there no signs of recurrence In twelve of the patients the roentgenograms show a small (less than 1 cm) pouch at the location of the diverticula In five patients the pouch is somewhat bigger and shows retention but no signs of stenoses In Table 1 and 2 the X ray investigation and the symptoms of the patients are described

Non-Operated Patients

The group of eight patients which for a variety of reasons was not operated on is illustrated in Table 3 In three of these patients the diverti

TABLE 3 *Patients with hypopharyngeal pouch which for different reasons has not been operated on*

Case no. Age year	Sex	Symptoms	X-ray	[illegible]	X-ray	[illegible]	[illegible] Symptoms	
1 [illegible]	♂	1/2 year [illegible]	[illegible]		[illegible]		[illegible]	
2 [illegible]	♀	1/2 year [illegible]	[illegible]		[illegible]		[illegible]	
3 [illegible]	♀	1/2 year [illegible]	[illegible]		[illegible]		[illegible]	
4 [illegible]	♂	1/2 year 1 [illegible]	[illegible]		[illegible]		[illegible]	
5 [illegible]	♀	1 year [illegible]	[illegible]		[illegible]		[illegible]	
6 [illegible]	♂	[illegible]	[illegible]		[illegible]		[illegible] died	[illegible]
7 [illegible]	♂	1/2 year [illegible]	[illegible]		[illegible]		[illegible]	
8 [illegible]	♀	1/2 year 1 [illegible]	[illegible]		[illegible]		1 [illegible]	

cula were f a size smaller than 1 cm in diameter The roentgenograms taken 8, 9 and 10 years later show no increase in the size of the diverticula, and the patients report that symptoms have not changed (cases nos 3, 4 and 8) Case no. 6 was followed during a ten year period. In spite of increasing symptoms and repeated recomm ndations he did not accept operation Finally he cam into the hospital in such a bad state that it was impossible to save him The other four cases in this group have been foll wed up to ten years. The symptoms as well as the size of the diverticulas have slowly increased.

DISCUSSION

Like most of the published follow-up investigations of esophageal diverticula this series i too small to allow any definite conclusions about the therapy of this disease However the relatively large number of operated patients who are not complet ly symtom-free has inspired us to see if it is possible to get from the literature on this subject definite opinions about the etiology and therapy of esophageal diverticula

Etiology

Intraluminal pressure registrations in the hypopharynx (Fyke & Code 19[illegible] Atkinson *et al* 1957) show that the cricopharyngeal muscle re-

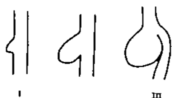


FIG. 1 The different stages of hypopharyngeal diverticula according to Lahey

mains in a state of tonic contraction. Under resting conditions there is a constant band of pressure of about 3 cm in length at the level of the cricoid cartilage. This pressure is around 30 cm of water with lower values in older subjects. In the act of swallowing this tonic contractions is for a moment actively inhibited and instead a negative pressure develops. Similar pressure registrations in patients with esophageal diverticula (Kodisek & Craemer 1961) show no significant difference either in tonic contraction or in the relaxing phase pressure. In spite of this several authors (Lewis & Edwards, 1962) state that the basis for the development of an esophageal diverticula must be a defect in neuromuscular coordination of hypopharyngeal muscles. They refer to an X-ray cinematographic study (Ardran, Kemp & Lund 1964) which in patients with hypopharyngeal pouch shows that the relaxing phase in the pressure band region is ended before all of the bolus has passed. However it must be impossible to judge from this investigation if the phenomenon described is primary or secondary to the development of the diverticula.

Therapy

Though there very seldom exist any difficulties in making the diagnosis of pharyngeal pouch there is often reason to discuss if and when the patient shall be operated on. If the diverticula is of small size, that is of stage I according to Lahey & Warren (1954) (Fig. 1) and the symptoms are light there is no reason to operate. The three cases described in our material with small diverticulas not increasing even after ten years observation speak in favour of this opinion.

Patients with bigger pouches, that is in stage II or III, mostly have moderate or severe symptoms. The risks for bad nutrition, regurgitation and aspiration pneumonia are increased and the indication for operation is clear. Two principally different kinds of operation exist. The endoscopial technique described by Dohlman & Matsson (1960) is proposed for old subjects judged to be bad operations risks. The lateral pharyngotomy and extirpation of the diverticula is today performed almost always as a one-step operation. The fact that the primary mortality of this operation is brought down to 1% (Clagett & Payne, 1960, Boyd 1961) is a good reason why most surgeons use this type of operation exclusively. Concerning the technique all authors agree that the diverticula should be dissected free from the muscular layer down to its neck and that the division is made in

the plane of the esophageal wall However different opinions exist as to whether the cricopharyngeal muscle should be divided or not. Clagett & Payne (1960) reports about 478 cases operated on with horizontal closure of the muscle layer Warren (1960) reports of 425 cases operated with division of the cricopharyngeal muscle There is no significant difference in number of recurrences between these two materials.

Reading different clinical reports one finds it very difficult to judge and compare the late results. The only existing objective method of control is the X ray investigation, but even this method has errors Further there is no exact correlation between the X ray pictures and the symptoms of the patients. There are patients who have clear roentgenologic recurrences but still have very mild symptoms. On the other hand there are patients with almost normal X-ray findings but moderate or even severe symptoms.

The patients who have been operated have for many years had more or less severe ymptoms. The relief these patients feel after operation is often judged as if they were free from symptoms. In fact very few of these patients are completely symptom-free. The "results" of the operation thus depend very much of the accuracy with which the investigator has penetrated the individual case.

CONCLUSIONS

In spite of many investigations the etiology of hypopharyngeal pouch is unclear

Patients with diverticulas in stage I according to the classification of Lahey shall be treated conservatively

All cases in stage II or III shall be operated The operation of choice is a one-stage lateral pharyngotomi excision of the pouch with or without divi ion of the cricopharyngeal muscle

There is need for an investigation of long term results in a large care fully f llowed material consisting of two groups, on operated with and the other without division of the cricopharyngeal muscle.

ZUSAMMENFASSUNG

L wird ber 31 Patienten mit Zenkerschen Divertikeln berichtet, die in den J hren 19.1–40 periert wurden sowie über 8 nicht perierte Fälle us derselben Zeit. Be den Patienten wurde in Symptomanamnese aufgen mmen sowie Rönt genbilder ngefertigt. In der Gruppe der operierten Patienten starben 6 Patienten nderen Kra kheiten 12 wurden beschwerdefrei, bei 9 bli ben leichte bei 4 stärkere S mptome best hend. Ei Patienti glaubt, das die Operation ihre Symptome nicht erbessert hat. Die Rö tgenergebnisse entsprechen nicht genau de Symptomen der Patienten. I der ichtoperierten Gruppe zeigen die Rönt genauf ahmen keine Grössenzunahme der kleinsten Di ertik la während iner 10-Jahresperiod E ist der Schlus gezogen worden, da die Ätiologi der Zenkerschen Divertik la unklar ist, d Fälle im ersten Stadi m konservati beha delt werd n sollen, Fälle im 2. und 3 Stadium sollten operiert werden

(mit einer lateralen Pharyngotomie mit Ausschälung des Divertikels mit oder ohne Durchtrennung des Musculus cricopharyngeus)

REFERENCES

ANDRAN, G M KEMP F H and LUND, W S 1964: Th etiology of the posteri pharyngeal diverticulum: a cin radi graphic study *J Laryng 78* 333.

ATKINSON M KRAMER, P WYMAN S M and INGELFINGER, F T 1957 The dynamics of swallowing I Normal pharyngeal mechanisms. *J Clin Inv st 36* 581

BOYD, D P 1961 Di erticula of the esophagus *Surg Clin. N Amer 41* 769

CLAGETT O T and PAYNE, W S 1960 Surgical treatment of pul ion di erticula of the hypopharynx: ne-st ge resection in 478 case *Dis Chest 37* 257

COVE. M 1959: Surgical treatment of the Zenker hypopharynx diverticulum *Pract Otorhin laryng* (Basel) *21* 129

DOHLM N G and MATTSSO O 1960 The ndoscopic operati n for hypopharyngeal di er ti ula a roentgencinemat graphic study *Arch Otolaryng* (Chic) *71* 744

FYKE, F E., JR., and CODE, C. F 1955: Resting and deglutition pressures in pharyngo-esophageal region *Gastroenterology 29* 24

KINCH. ER, J A., 1958 Th m t ctl ity f the cricopharyng us muscl *Laryngoscope 68* 1119

KODISEK, J and CRAEMER, B 1961 A study of ph ryngeal pouches. *J Laryng 75*, 406.

KORKIS, F B 1958 The aetiology di gnosi nd surgical treatment of pharyng al di erticula *J Laryng 72* 509

LAHEY F H an l WARREN, K W 1954: Esoph geal di erticula. *Surg Gyn. Obst 98* 1

LEGLER, L 1960 L t w l h n V raussetzung n ist di endo k pische Operation Zen-kersche Di ertikeln zu verantw rt n? *Z La yng Rhinol Otol 39* 405

LEWIS, R S and EDWARDS, W G 1962: The tre tm t f pharynge l diverticula Brit J Surg 50 1

NEGUS, V E., 1950 Ph ryngeal di rticula observation on their evolutio and treat m nt *B t J Surg 38* 129

PAYNE, W S nd CLAGETT O T 1965: Pharyng l d esoph g al div rticul *Curr Probl Su g* pe 1

RÜEDI, L nd RÜEDI, P 1963 Die chirurgische Beha dl g des Ze kerschen Di ertikels. Acta Ot laryng (Stockh) Suppl. 183 132

UN ERECHT K 1963: Hi t re H popharyn di ertik l (Berendes, J (ed.)) *Arch Ohr Nas K hlk pfh ill* ol 2/T 1 489

WARREN K. W 1960: S m technical considerations in the man gem nt f pharyngo esophageal di erti ulum *Su g Clin N Am 40* 633

D pt f Ot laryng logy Sahlgrenska Sjukhu et University of G th nburg Göt borg Swed n

R cei d Oct be 1 1966

Acta oto-laryngologica 64 37–44 (1967)

BACTERIAL FLORA IN CHRONIC, PURULENT MAXILLARY SINUSITIS

J. KINNMAN, CHANG WON LEE and SEUNG HAHM PARK
Seoul, Korea

From the Department of Otolaryngology (Head: J. Kinnman) and the Department of Microbiology (Head: Seung Hahm Park) National Medical Center, Seoul

A series of 100 patients (150 sinuses) with chronic, purulent maxillary sinusitis experienced during the period September–May 1966, has been studied. Hemophilus influenzae was found in 48.7 per cent of all sinuses and Diplococcus pneumoniae in 29.3 per cent. These two dominating bacterias were usually found in pure culture or together. The presence of Staphylococcus is regarded as an indicator of contamination. Staphylococcus aureus in pure culture was found in only 5 sinuses, and in 8 sinuses this bacteria was an additional finding in a dominating growth of Hemophilus influenzae. Diplococcus pneumoniae was evenly distributed during the seasons, while Hemophilus influenzae dominated during the winter and spring seasons, but almost disappeared during autumn. Only patients filling the criteria of chronic, purulent maxillary sinusitis were included. More than half of the patients were younger than 20 years and only 7 over 40 years. The investigation demonstrates that Hemophilus influenzae and Diplococcus pneumoniae are dominating in the younger age groups. Seasonal variations and the dominance of certain bacterias in different age groups must be regarded when conclusions are made about the etiology of maxillary sinusitis.

INTRODUCTION

The bacterial flora in maxillary sinusitis has been the subject of many investigations, and various techniques have been employed to obtain representative specimens with a minimum of contamination. The variation in bacteriological findings reported in different series is probably to a certain degree a consequence of different sampling methods (Lystad, Berdal & Lund Iversen, 1964). However the possibility remains of seasonal and climatic variations, as well as the predominance of certain bacteria in different age groups.

The purpose of this study has been to find the most common etiologic bacteria in chronic purulent maxillary sinusitis among our patients, and, if possible, to demonstrate variations in different age groups and during different seasons.

TABLE 1 *Age and sex distribution*

	<11	11–20	21–30	31–40	41–70	Total
Male	2	33	13	5	3	56
Female	1	19	17	3	4	44
Total	3	52	30	8	7	100

MATERIAL AND METHODS

The material consists of 100 unselected patients with long standing purulent rhinorrhea and other nasal symptoms seen in the Out Patients Department during a period from September 1965 to May 1966 The duration of the symtoms was more than 12 months, and pus or mucopus came out by diagnostic puncture in all cases. In 50 cases the sinusitis was bilateral and accordingly 150 sinuses have been studied

The age and sex distribution is shown in Table 1 More than half of the patients were younger than 21 years and only 7 over 40 years. In order to study possible seasonal variations in the bacterial flora the material was classified in 3 groups Group 1 comprises patients seen during September October and November group 2 those seen during December January and February and group 3 those seen during March April and May (Table 2 and Fig 1) The distribution of bacteriological findings in different age groups was also studied (Table 3)

The samples for bacteriological examination were taken using the technique described by Lumio & Oker Blom (1957) and by Kortekangas (1964) After local anesthesia the nasal cavity was carefully sucked clean the maxillary sinus was punctured and a lump of pus was collected from the saline lavage into a sterile dish The specimens were cultured on blood agar media within 12 hours, and, if indicated, direct smears were also studied Only the samples taken at the first diagnostic puncture were examined No attempt was made to correlate the bacteriological finding to the subsequent clinical course or the sensitivity test In no case was chemotherapeutica or antibiotica used

RESULTS

In the specimens from 19 sinuses there was no growth of bacteria (12 7 per cent) Hemophilus influenzae was found in pure culture in 48 of the 150 sinuses, and in a further 25 sinuses together with other bacteria, mainly Diplococcus pneumoniae. Hemophilus influenzae obviously was the most common pathogen bacteria in this study and was found in 48 7 per cent of the sinuses Diplococcus pneumoniae was found in 29 3 per cent of all sinuses, Staphylococcus aureus in 9.3 per cent and Staphylococcus albus

Table 2 *Results of bacteriological analyses in different seasons*

Seasonal group →	Group I, Sept./Oct./Nov		Group II, Dec./Jan./Febr		Group III Mar./Apr./May		Unilateral result		Bilateral result		Total number of sinuses
Bacteriological finding	M	F	M	F	M	F	M	F	M	F	
Hemoph. infl.	1	1	22	10	8	6	15	11	8	3	48
Dipl. pneum.			8	1	2	5	6	2	1	2	14
Staphyl. aureus			1		1	1	2	1			3
Staphyl. albus				1	1		1	1			2
Streptoc. viridans		1	1				1	1			2
Neiss. pharyng.					1		1				1
Staphyl. aureus											
Neiss. pharyng.						1		1			1
Staphyl. aureus											
Klebs. aerog.				2						1	2
Dipl. pneum.	6	2	10	3	6	2	10	3	5	2	27
Neiss. pharyng.						1		1			1
Klebs. aerug.					1		1				1
Pseud. aerug.					1		1				1
Staphyl. aureus	1	1	1		1	1	3	2			5
Streptoc. viridans				1				1			1
Streptoc. hemolyt.		1						1			1
Alcal. fecal						1		1			1
Staphyl. albus	1	1	1				2	1			3
Neiss. pharyng.				2						1	2
Spir. and Fusif.					1		1				1
Diphteroids		1						1			1
Streptoc. viridans	1						1				1
Streptoc. viridans		1		1				2			2
Neiss. pharyng.			1				1				1
Diphteroids				1				1			1
Streptoc. hemolyt.											
Diphteroids											
Alcal. fecal	1						1				1
Neiss. pharyng.			1			1	1	1			2
Klebs. aerug.		1						1			1
Klebs. rhinoscl.					2				1		2
Moraxella pl.		2								1	2
No growth	2	2	1	4	5	4	8	9		1	19
Total number of sinuses	12	13	45	26	29	23	56	42	15	11	150

TABLE 3 *Results of bacteriological analyses in different age groups*

Age groups years	<11		11–20		21–30		31–40		>41		Summa		
Bacteriological finding	M	F	M	F	M	F	M	F	M	F	M	F	Total
Hemoph. infl.	1		23	5	5	0		3			31	17	48
+ Dipl. pneum			6	2	2	2				2	8	6	14
+ Staphyl. aureus			2	1								1	3
+ Staphyl. albus			1			1					1	1	2
+ Streptoc. viridans			1							1	1	1	2
+ Neiss. pharyng.			1								1		1
+ Staphyl. aureus + Neiss. pharyng.				1								1	1
+ Staphyl. aureus + Klebs. aerog.				2								2	2
Dipl. pneum	2	2	10	3	1	2	4		3		20	7	27
+ Neiss. pharyng.				1								1	1
+ Klebs. aerog.			1								1		1
+ Pseud. aerug.									1		1		1
Staphyl. aureus			1	1	2	1					3	2	5
+ Streptoc. viridans				1								1	1
+ Streptoc. hemolyt.						1						1	1
+ Alcal. fecal				1								1	1
Staphyl. albus				1	1				1		2	1	3
+ Neiss. pharyng.				1	1			1				2	2
+ Spir. and Fusif.					1						1		1
+ Diphteroids						1						1	1
+ Streptoc. viridans					1						1		1
Streptoc. viridans				1		1						2	2
+ Neiss. pharyng.					1						1		1
+ Diphteroids				1								1	1
Streptoc. hemolyt. + Diphteroids + Alcal. fecal	1										1		1
Neiss. pharyng.	1			1							1	1	2
Klebs. aerog.				1								1	1
Klebs. rhinosc.			2								2		2
Moraxell. pir.										2		2	2
No growth			3	5	3	5	2			1	8	11	19
Total number of sinuses	5	2	53	28	17	23	6	5	5	6	80	61	150

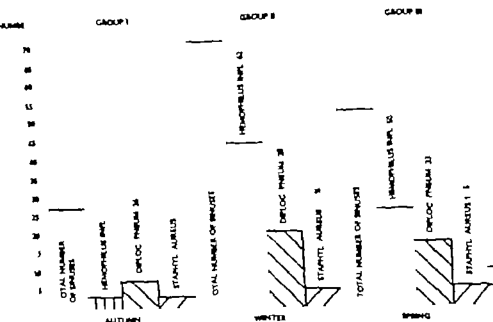


Fig. 1 This figure demonstrates the total number of sinuses in which the more important bacteria were found during the different seasons. The percentual distribution is also indicated for each bacteria.

in 6.3 per cent Streptococcus viridans and Neisseria pharyngis were both found in 5.3 per cent, Streptococcus hemolyticus however only in 1.8 per cent. It must also be stressed that the dominating bacteria, Hemophilus influenzae and Diplococcus pneumoniae, were mainly found either in pure culture or together.

The percentual distribution during the different seasons is shown in Fig. 1. Diplococcus pneumoniae is rather evenly distributed over the seasons, while Hemophilus influenzae is dominating during the winter and spring, but almost disappears in the autumn. The "sinusitis-season" among our patients lasts from September to May and during the summer we have very few cases, so a comparison could accordingly not be done. Staphylococcus aureus is rather evenly distributed over the seasons.

There are astonishingly few chronic maxillary sinusitis in the age group above 31 years in our material. It must be pointed out that sinusitis in combination with maxillary cancer are not included, and this disease is rather common in Korea. The age group below 11 years is also small partially caused by the fact that maxillary puncture in some cases could not be performed in local anesthesia, and smears taken at later endonasal trepanation not were considered comparable. It is however reasonable to state that in our material Hemophilus influenzae is the most common etiologic factor in chronic purulent maxillary sinusitis in the age groups 11–30 years.

DISCUSSION

(1) *Method*

Staphylococcus and other contaminants from the nose, for example Neisseria and Diphteroids, may overgrow and mask the presence of causal microbes contained in the sinus secretions (Berdal & Lund-Iversen, 1964) Accordingly a small number of Staphylococcus is a good indicator of a proper technique Staphylococcus was found in pure culture in only 5 sinuses In 8 of the sinuses, where Staphylococcus appeared, Hemophilus influenzae was the dominating bacteriological finding Lystad, Berdal & Lund-Iversen (1964) recommended samples taken for bacteriological examinations by puncture of the maxillary sinus and aspiration of the secretion without instillation of saline this, however was possible in only somewhat more than half of their cases. In our series exactly the same *technique has been used in all patients, and the examiner has always been* the same If the finding of Staphylococcus is regarded as contamination our results are comparable with those of other authors (Table 4) Kim (1959) found 64 3 per cent Staphylococcus in a series of 28 sinuses in Korea and Hohenwald (1965) 30 per cent Staphylococcus in pure culture in a series of 96 sinuses in Germany

(2) *Results*

In Table 4 our findings are compared with those of other authors, who have used a fairly comparable technique It must be stressed, however that the age distribution is not quite comparable in the different materials, and that the seasonal variations have not been considered in previous series It is quite possible that if all our 100 patients had been examined during the autumn season, Diplococcus pneumoniae would have been dominating in the whole series instead of Hemophilus influenzae (Fig 1) It is, however *obvious that in almost all previous series Diplococcus pneumoniae* and Hemophilus influenzae are dominating Bjuggren *et al* (1953) examined 54 sinuses in children 3–7 years old and found 55 6 per cent Diplococcus pneumoniae and 31 6 per cent Hemophilus influenzae stressing that Hemophilus influenzae was often found in the sinus without positive finding in the nasal smears. The marked tendency of Hemophilus influenzae to remain in the paranasal sinuses long after the acute stage of an upper respiratory airway infection is characteristic, and this bacteria has also been demonstrated to be responsible for recidives of acute otitis media in a high percentage (Bjuggren & Tunevall 1962) Bjuggren *et al* (1953) also pointed to the fact that when bacteria were found in the sinus, the tendency to antibody formation was high, and concluded that the antibody formation against the common pathogenic bacteria originates from sinus processes, but that the simple catharral infection without complications from the sinuses, has little influence as regards this It is accordingly a temptation

Acta oto-laryngologica 64: 46–61 (1967)

OXYGEN CONSUMPTION IN NORMAL AND KANAMYCIN DAMAGED COCHLEAE

O. Mizukoshi and J. F. Daly
New York, N.Y., U.S.A.

From the Department of Otorhinolaryngology, New York University School of Medicine, New York

The oxygen consumption of each turn of the guinea pig cochlea was measured with a differential type ultra-microrespirometer. The basal portion of the cochlea had greater rate of oxygen consumption per milligram of tissue than the apex. The rate of oxygen consumption was found to be dependent on the substrate and potassium concentration. Kanamycin damaged cochleae had decreased rate of oxygen consumption.

INTRODUCTION

The inner ear is dependent upon oxygen metabolism. A decrease in the oxygen supply to the inner ear results in a decrease in the endocochlear potential, cochlear microphonic and action potential of the eighth nerve (Davis, 1960). Some attempts to measure oxygen consumption of the cochlea have been reported by Mizukoshi (1960) and El M fty & El Serafy (1966) with the use of the Warburg respirometer. The Warburg apparatus was not sensitive enough to measure the oxygen consumption of a segment of the cochlea. Nakai (1963), Kawamoto et al. (1963) and Meyer zum Gottesberge, Rauch & Koburg (1965) used the Cartesian Diver method to measure oxygen consumption. The Cartesian Diver method had sufficient sensitivity but an accurate weight of the specimen could not be obtained.

This study presents data on the oxygen consumption of the various turns of the cochlea in normal and kanamycin damaged guinea pigs. The oxygen consumption was measured with a differential type of microrespirometer.

METHOD

An all glass differential type of ultra-microrespirometer improved by Cunbaum et al. (1955) and produced by Microchemical Specialities Company (MISCO) was used to measure oxygen consumption. This respirometer consisted of two cells and a connecting capillary. Type "B" cells and

Present address: Dept. of Otorhinolaryngology, Kyoto Prefectural University of Medicine, Kyoto, Japan.

DISCUSSION

(1) *Method*

Staphylococcus and other contaminants from the nose for example Neisseria and Diphteroids, may overgrow and mask the presence of causal microbes contained in the sinus secretions (Berdal & Lund-Iversen 1964) Accordingly a small number of Staphylococcus is a good indicator of a proper technique Staphylococcus was found in pure culture in only 5 sinuses. In 8 of the sinuses, where Staphylococcus appeared Hemophilus influenzae was the dominating bacteriological finding Lystad, Berdal & Lund-Iversen (1964) recommended samples taken for bacteriological examinations by puncture of the maxillary sinus and aspiration of the secretion without instillation of saline this, however was possible in only somewhat more than half of their cases In our series exactly the same technique has been used in all patients, and the examiner has always been the same If the finding of Staphylococcus is regarded as contamination, our results are comparable with those of other authors (Table 4) Kim (1959) found 64 3 per cent Staphylococcus in a series of 28 sinuses in Korea and Hohenwald (1965) 30 per cent Staphylococcus in pure culture in a series of 96 sinuses in Germany

(2) *Results*

In Table 4 our findings are compared with those of other authors, who *have used a fairly comparable technique. It must be stressed, however* that the age distribution is not quite comparable in the different materials, and that the seasonal variations have not been considered in previous series it is quite possible that if all our 100 patients had been examined during the autumn season, Diplococcus pneumoniae would have been dominating in the whole series instead of Hemophilus influenzae (*Fig 1*) *It is, how* ever obvious that in almost all previous series Diplococcus pneumoniae and Hemophilus influenzae are dominating Bjuggren *et al* (1953) examined 54 sinuses in children 3–7 years old and found 55 6 per cent Diplococcus pneumoniae and 31 6 per cent Hemophilus influenzae, stressing that Hemophilus influenzae was often found in the sinus without positive finding in the nasal smears The marked tendency of Hemophilus influenzae to remain in the paranasal sinuses long after the acute stage of an upper respiratory airway infection is characteristic, and this bacteria has also been demonstrated to be responsible for recidives of acute otitis media in a high percentage (Bjuggren & Tunevall 1962) Bjuggren *et al* (1953) also pointed to the fact that when bacteria were found in the sinus, the tendency to antibody formation was high and concluded that the antibody formation against the common pathogenic bacteria originates from sinus processes, but that the simple catharral infection without complications from the sinuses, has little influence as regards this. It is accordingly a temptation

TABLE 3. *QO of each turn of the normal cochlea.*

Medium Modified Krebs Ringer phosphate buffer (potassium 140/meq/l)
Substrate 0.2% succinate.

Animal Number	Apex and 4th turn	3rd turn	2nd turn	Basal turn
1	4.0	8.2	16.3	14.4
2	8.6	12.8	23.6	23.2
3	6.7	10.5	12.5	13.6
4	12.1	18.6	19.2	21.3
5	10.5	18.8	24.3	20.9
6	9.6	14.3	19.7	16.0
7	11.2	15.1	22.8	21.8
8	8.5	18.0	18.2	17.0
9	7.2	12.9	15.0	15.3
10	14.9	14.9	19.0	18.9
Mean	9.5	14.2	19.1	18.1

O μl mg dry weight/60 min.

The results in the Kanamycin guinea pigs are shown in Tables 5 6 and The QO of the cochlea were measured only in the 140 meq/l potassium KRPB solution with the succinate substrate as preliminary studies showed that the QO_2 would be too small to allow accurate measurements in the other mediums. The mean QO_2 value of Kanamycin damaged cochleae decreased with increasing doses of Kanamycin. The normal value of the

TABLE 4 *QO of each turn of the normal cochlea*

Medium Modified Krebs Ringer phosphate buffer (potassium 140/meq l)
Substrate 0.2% glucose.

Animal Number	Apex and 4th turn	3rd turn	2nd turn	Basal turn
1	3.0	4.8	6.0	10.1
2	6.2	8.1	7.8	8.1
3	5.2	7.3	10.9	15.4
4	1.8	4.8	6.8	9.3
5	9.6	9.8	12.1	12.1
6	7.3	8.5	9.3	11.6
7	3.6	8.3	8.6	10.1
8	2.8	4.6	7.8	8.5
9	4.0	7.3	12.6	9.2
10	3.1	4.6	9.3	8.1
Mean	4.	6.5	8.6	10.1

O_2 μl/mg dry weight/60 min.

TABLE 2 QO_2 *of each turn of the normal cochlea*

Medium: Krebs Ringer phosphat buff Substrate: 0.2% glucose

Animal Number	Apex and 4th turn	3rd turn	2nd turn	Basal turn
1	2.8	4 1	8.8	8.7
2	0.3	7.2	8.2	6.5
3	4 5	4 6	10.1	12.3
4	2.0	3.1	4 1	6.8
5	1 7	—1	4 8	9.2
Mean	3.5	4.2	7.2	8.7

O µl/mg dry weight/60 min

0 001 mg in a range of 0 005 mg to 0.5 mg The value of oxygen consumption of each turn of the membranous cochlea was expressed as microliters of oxygen per milligram of cochlea dry weight per sixty minutes. This was called the QO_2.

QO determinations were made in 35 normal and 22 Kanamycin damaged guinea pigs The Kanamycin damaged group was composed of three subgroups in which 400 mg of sulfated kanamycin per kg of body weight was administered intraperitoneally in daily doses for five days (Group B) ten days (Group C) and twenty days (Group D)

RESULTS

When succinate was used as a substrate the mean QO_2 was 7.5 in the fourth turn and apex 9 1 in the third turn, 12 8 in the second turn and *12.5 in the basal turn (Table 1) The mean QO_2 of the basal portion of the* cochleae was higher than that of the apical portion The mean QO value was lower when glucose was used instead of succinate but the mean QO_2 of the basal portion of cochlea was still greater than the apical portions (Table 2)

The mean QO_2 increased with the use of the high potassium solution This was found when either glucose or succinate were used as a substrate (Tables 3 and 4) The mean value of the QO_2 was highest when the high potassium KRPB was used with the succinate substrate. In this group the mean QO_2 was 9 5 in the fourth and apical turns, 14.2 in the third turn 19 1 in the second turn and 18 1 in the basal turn The mean QO_2 of the basal portion of the cochlea was *about twice that of the apical portion* When a combination of the high potassium KRPB and the glucose substrate was used, the *mean* QO_2 was 4 7 in the fourth turn and apex, 6 5 in the third turn, 8 0 in the second turn and 10 1 in the basal turn This mean QO_2 was lower than that of high potassium KRPB and succinate but higher than that of normal KRPB and glucose

TABLE 7 QO of *each turn of the Kanamycin damaged cochlea*

Medium Modified Krebs Ringer phosphat buffer (potassium 140/meq/l)
Substrat 0.2% succinate (R)

Group D (Kanamycin 400 mg for 20 days)

Animal Number	Apex and 4th turn	3rd turn	2nd turn	Basal turn
1	4.8	6.1	6.7	7.4
2	1.8	1.9	2.9	3.9
3	4.9	5.2	6.3	7.2
4	2.3	3.8	5.1	5.3
5	3.6	5.3	6.6	6.2
6	6.2	6.9	7.9	8.1
7	2.8	4.5	5.0	5.5
Mean	3.8	4.8	5.8	6.2

O μl mg dry weight/60 min.

DISCUSSION

The respiratory activity (QO) of the four turns of the guinea pig cochlea was measured with the differential type of ultra microrespirometer. The measures of the QO_2 were more accurate than those done with other method because the sensitivity of the ultra microrespirometer was in the proper range to measure the variation of the QO_2 of the cochleae and the dry weight of cochlear tissues could be obtained. Previous studies either failed to report the dry weight of the specimen (Mizukoshi, 1962 Kawamoto *et al* 1963 Meyer zum Gottesberge, Rauch & Koburg, 1965 Nakai, 1963) or probably reported weights which included neural tissue El Mofty & El-Serafy (1966)

The larger mean QO obtained in the present studies when succinate as opposed to glucose was used as a substrate indicated that the value of the



FIG. 3. Respiratory activity (QO_2) after Kanamycin injection A, normal; B, Kanamycin injected for 5 days; C, Kanamycin injected for 10 days; D, Kanamycin injected for 20 days.

TABLE 5 *QO_2 of each turn of the Kanamycin damaged cochlea*

Medium: Modified Krebs Ringer phosphate buffer (potassium 140/meq/l)
Substrate: 0.2% succinate

Group B (Kanamycin 400 mg for 5 days)

Animal Number	Apex and 4th turn	3rd turn	2nd turn	Basal turn
1	6.5	9 0	12.8	12.7
2	5.3	12.4	15.3	15.1
3	9 9	12 1	14 0	13.0
4	8.2	14.5	13.1	11 0
5	6.8	11.9	12.2	12.8
6	7.6	10.3	13.3	13.8
7	6.2	9.3	0.9	8.9
Mean	7.2	11 4	13 0	12.5

O μl/mg dry weight/60 min

mean QO_2 of each turn was defined as 100% The mean QO_2 of the Kana mycin damaged cochleae was expressed as a percent of the normal (Fig 3) After five days (Group B) the mean QO_2 was 75 7% in the fourth turn and apex 80 0% in the third turn, 66 8% in the second turn and 68.8% in the basal turn *The mean QO_2 for each turn of the group injected for ten days* (Group C) starting at the apex was 70 7% 58 7% 47 2% and 47 6% In the group injected for twenty days (Group D) the mean QO in each turn starting at the apex was 39 4% 34 0% and 34.2%

TABLE 6 *QO_2 of each turn of the Kanamycin damaged cochlea*

Medium Modifi d Krebs Ri ger phosphat buffer (potassi m: 140/meq/l)
Substrat : 0.2% uccinate

Group C (Ka amycin 400 mg fo 10 days)

Animal N ber	Apex and 4th turn	3rd turn	2nd turn	Basal t rn
1	5 4	6.9	.5	7 1
2	8.5	10 1	11 0	10 4
3	5 4	6.4	7 1	6.3
4	7.2	0 0	9 7	10 1
5	6.9	8 4	0.2	8 1
6	6 1	7 7	8 1	8.6
7	8.3	9 9	10 6	9 5
8	6.4	8.2	8.9	9 0
Mean	6 8	8.3	9 0	8.6

O μl/mg dry weight/60 min

Spitze. E wurde gefunden dass die Höhe des Sauerstoffverbrauches vom Substrat und der Kaliumkonzentration abhängig ist. Die durch Kanamycin geschädigten Schnecken hatten einen herabgesetzten Sauerstoffverbrauch.

REFERENCES

Békésy G. von, 1958. Pendulums, travelling waves and the cochlea. *Laryngoscope* 68 317

D rs, H 1960 Ad nces in the neurophysiology and euroanatomy of the cochlea. *J Acoust Soc Am* 31 1377

D all, A. J and Wersäll, J 1964 Sit of action of strept mycin pon inner ea sensory cells. *Act Otolaryng* (Stockh.) 57 581

El-Mofty A nd El-Serafy S 1966 The effect of ototoxic tibiotics on the oxygen ptake of the membranous cochlea. *A n. Otol.*, 75, 216.

Goldstein A nd Mizukoshi, O.: In preparation.

(vas n, B W Sweet, B V Schultz, A R and Kirk, P L., 1954 Determination of oxygen pt ke by tissue grown in glass differential microrespirometer *Microchimica A ta* 5–6, 1069

I ka, T The ev luation f adenosine triphosphat se in the stria vasculari nd piral lig ment f the normal guinea pig. *Laryngoscope* I press.

Im a, T Mizukoshi, O nd Dal J F Possible effect of rious ototoxic drugs pon the ATP-hydrolysi g sy tem i the stria ascularis and spiral ligament f the guinea p g. *Laryngoscope* I press.

K ahara, S Kaw oto, K K rao, Y Kataoka, K Hosoi, K., nd W ke, K., 1965 Experiment l studies on the t ic effect of Kanamyci on the inner ea *VIII International Congress of Otorhinolaryng* Exh bition, Tokyo

Kaw moto, K T kama i, k., Kon tsuda, Y Kaneko, Y Ka za i, I nd W ake, S., 1963 A histochemical study on the labyrinth. *T hok Med J* 67 134

K a, M T ra, H T amura, H., nd H nada, Y 1963 Histochemical study of ucci ic dehydrogenase f guinea pig cochlea Audiology (J p.) 6, 58.

Koide Y H a, A nd H ano, R 1966 V lnerabil ty of the org f Corti i poisoning A t Otolaryng (Stockh 61 332

M rum Gottschewski, A R cn S., nd Kom no, E., 1965 Unterschiede i Metabolismus der inzelnen Schneckenwindungen. *Act Otolaryng* (Stockh.) 59 116.

Mizukoshi, O 1959: Some metabolic Reaction i the cochlea *Jap. Jou Otol* 62 ppl 2

Mizukoshi O nd D J F Chemical acti ity at the reticula membrane of org f Cort I preparation

N ka Y 1963 Oxygen consumption of stria ascularis nd the piral ligament f the guinea pig *Jap Jou Otol Tokyo* 66 1103

N na F 1965 Susceptibility of the ochlea t sound pos re nd Kanamyci *VIII I ternational Congress f Otorh nolaryng* Tokyo.

R ners C L., nd Wever W F 1960 *N ral Mechani ms in Auditory and Vesti ul r Systems* C C Thomas, Spri gfield.

Sm C A Low O H nd R M. L., 1954 Electrolytes f l byrinthine fl ids. *Laryngoscope* 63 141

Spoendlin, V H., nd Balogh, K., Jr., 1963 Licht und lektronenmikroskopische Darst llung on Dehydrogen sen i der Schneck nach Intra it ler I k bation. *Pract t hinol rhing* Basel 25, 139

re H nd Kirk, P L., 1948 A ersatile microrespirometer for routine se *J Gen. Physiol* 31 329

Stiver H R cn S., Sch., H nd Lauer, F 1966 Untersuchungen über die Ursache

mean QO_2 was dependent upon the substrate being used The histochemical findings (Vosteen 1956 and 1961 Mizukoshi 1962 Kawata *et al* 1963 Nakai 1963) of wide spread succinic dehydrogenese activity in the cochlea may be related to the large mean QO_2 when succinate was used as a substrate The QO_2 was also found to increase when the concentration of the potassium was increased A possible mechanism to account for this would be the effect which the high potassium concentration has upon the cell membrane Studies by Goldstein & Mizukoshi (in preparation) have shown that the base of the hair cell swells and the cell membrane disintegrates causing the contents of the hair cell to be exposed to the substrate. This would cause an increase in the availability of the substrate to the mitochondria of the cells with a probable increase in the QO_2.

The present data show the mean QO_2 was greater in the basal segments than in the apical segments This data was qualitatively consistent with the work of (Nakai 1963 Kawamoto *et al.*, 1963 Meyer zum Gottesberge Rauch & Koburg 1965) It is generally agreed that there is a graded continuum of oxygen consumption with the basal portion being more active than the apex This pattern of aerobic metabolism is consistent with the increased work load of the basal portion of the cochlea which vibrates in response to a larger spectrum of frequencies (Rasmussen & Windle 1960)

There was a decrease in the mean QO_2 of the cochlea in the Kanamycin injected guinea pigs These results parallel the histochemical findings of a decrease in succinic dehydrogenase activity in Kanamycin injected guinea pigs (Katagiri *et al* 1965 Nakamura 1965 Koide, Hata & Hando, 1966) The decrease in the mean QO_2 in each turn of the cochlea was not constant with an increase in the Kanamycin dosage A small amount of Kanamycin (Group B) resulted in a moderate decrease in the mean QO_2 of the whole cochlea (Fig 3) A moderate amount of the Kanamycin (Group C) resulted in a greater decrease of the QO_2 in basal turn than the apical turn This is a pattern of damage which could suggest a high tone hearing loss. The larger doses of Kanamycin (Group D) resulted in a large decrease of the mean QO_2 of the whole cochlea

Kanamycin is concentrated in the perilymphatic and endolymphatic fluids Stupp *et al* (1966) This characteristic of the drug must augment its effect in the inner ear Kanamycin may cause damage to the inner ear by decreasing the QO_2. It is also possible that Kanamycin effects the integrity of the cell membrane as a similar drug Streptomycin, disrupts cell membranes in the ear and Kanamycin inhibits ATPase (Mizukoshi, in preparation) a cell membrane enzyme

ZUSAMMENFASSUNG

Der Sauerstoffverbrauch in jeder Windung der Meerschweinchenschnecke wurde mit einem Differential Mikrorespirometer gemessen Der basale Anteil der Cochlea hatte einen grösseren Sauerstoffverbrauch pro mg Gewebe als die

Acta oto-laryngologica 64 56–64 (1967)

EFFECT OF THE OSMOTIC PRESSURE OF SOLUTIONS APPLIED TO THE COCHLEA OF GUINEA PIGS AND CATS ON COCHLEAR POTENTIALS

H. Sohmer and M. Feinmesser

Jerusalem, Israel

From the Rogoff Laboratory of Physiology and the Department of Otolaryngology, Hebrew University-Hadassah Medical School, Hadassah University Hospital

In experiments involving cochlear application of drugs, one must consider the osmotic pressure of the solutions applied. Intracochlear injection in guinea pigs, and round window application in cats, of solutions with a tonicity of twice that of ordinary Ringer's solution causes a depression of the action potential. Solutions with tonicities of up to 20 times that of ordinary Ringer's solution did not have any effect on the cochlear microphonic potentials. An attempt is made to analyse these results.

Several drugs used in medical practice have been found to be ototoxic when applied systemically. This has led to experimental work designed to test their toxicity. Techniques, involving the intracochlear injection (guinea pig) of the drug dissolved in a suitable medium (Ringer's solution) or the round window application (cats) of the drug (in distilled water or Ringer's solution) have been used to test the safety of drugs given systemically or locally to the ear. The effect of these drugs on the cochlear potentials, evoked by suitable sound stimuli, has been observed and their cochlear toxicity thereby evaluated (Cutt 1963, Rahm *et al.* 1959, Kohonen, Hata & Hando, 1966, Feinmesser & Sohmer 1965, Hennebert & Fernández, 1959). However, several of these studies have not considered the effect of the osmotic pressure of the solution applied, independent of any toxic nature of the dissolved drug. Therefore the purpose of the present study was to investigate the effects of hypotonic and hypertonic concentrations of non-toxic substances on the cochlear potential when applied directly to the cochlea so as to try to set upper and lower limits to the concentration of solutions which may be applied directly to the cochlea when evaluating the cochlear toxicity of a drug.

This work was supported by a grant from the Joint Research Fund of the Hebrew University Hadassah Medical School, Jerusalem, Israel.

der spezifisch totoxischen Wirkung der basischen Streptomyces-Antibiotika unter besonderer Berück ichtigung des Kanamycin *Acta Otolaryng* (Stockh) *61* 435.
VOSTEEN K H 1956: Die Darstellung der Bernsteinsäuredehydrogenase in der Schnecke des Meerschweinchens *Arch Ohren Nasen Kehlk pfheilk* *168* 295
— 1956 Die Tetrazolreaktion in d r Meerschweinch nschnecke nach Blockierung der Zellatmung durch Kaliumyanid *Arch Ohren Nasen Kehlkopfh ilk* *169* 415
— 1961 Neue Aspekte zur Biol gie und Pathologie des Innenohres *Arch Ohren Nas n. Kehlkopfheilk* *178* 1

O Mi ukoshi M D
Dept of Oto hinolaryngology
Kyoto Furitsu University of Medicine
Kyoto Japan

Received November 28 1966

TABLE 2. *Composition and tonicities of solutions applied to the round window of cats.*

No.	Solution: Material	Solution: Dissolved in	Tonicity	Number of experiments
1	Sucrose	Water	1	18
2	Ringer's	—	1	20
3	Choline chloride*	Water	1	2
4	Sucrose	Water	2	8
5	Sucrose	Ringer's	2	6
6	Choline chloride	Water	2	2
7	Sucrose	Water	3	2
8	Sucrose	Ringer's	3	2
9	Sucrose	Water	4	4
10	Sucrose	Water	5	3
11	Sucrose	Water	10	1
12	Sucrose	Water	20	1
13	NaCl*	Water	20	1
14	Water	—	0	3
15	Sucrose	Water	0.5	2

Isotonic sucrose solution 113 mg/ml. Isotonic choline chloride solution = 23 mg/ml; isotonic sodium chloride solution 9 mg/ml.

the cochlea through the scala tympani and then flowed back via the scala vestibuli, to exit through the hole drilled for the electrode in the basal turn. The volume injected (ca. 0.1 ml) was always greater than the volume of the perilymphatic spaces (Wever & Lawrence, 1954) and was thus sufficient to completely replace the perilymph with injected fluid.

Sucrose in various concentrations was used to provide non-toxic solutions of different osmotic strengths. It was dissolved in ordinary mammalian Ringer's solution (NaCl 0.9%, KCl 0.042%, CaCl 0.024%, $NaHCO_3$ 0.020%) which has also been shown to be non-toxic (Tasaki & Fernández, 1952). Ringer's solution diluted with distilled water was also used. An isotonic solution has been assigned the value of tonicity 1 so that hypertonic solutions are multiples of 1 and hypotonic solutions, fractions (Table 1).

Cats

Twenty-six cats were anesthetised with pentobarbital followed by surgical exposure and opening of the bulla. A ball-tipped silver wire electrode, insulated except for the tip, was then placed on the bony ridge alongside the round window. The round window niche was left clear for the application of test solutions. This was found necessary when it was observed

METHODS

These experiments were performed on the experimental animals in common use in auditory physiology experiments, cats and guinea pigs.

Guinea Pigs

Eleven guinea pigs were anesthetised by an intraperitoneal injection of Dial in Urethane® (0.5 cc/kg) After surgically exposing the auditory bulla and the cochlea within it 2 small holes (50–100 μ in diameter) were drilled in the basal turn, one in the scala vestibuli for electrode insertion and one in the scala tympani for intracochlear injections. The active electrode was a nichrome-steel wire 25 μ in diameter insulated except for the tip This was inserted into the hole in the scala vestibuli A small "bead" of insulating resin on the wire prevented the insertion of the electrode too deep into the cochlea The indifferent electrode was connected to the animal head holder The responses were amplified by a Tektronix 122 preamplifier displayed on a Tektronix 502 oscilloscope and photographed with a Grass Kymograph Camera.

Click acoustic stimuli were used so that the cochlear responses (cochlear microphonic and action potentials) to the same acoustic stimulus could be simultaneously observed The clicks (60–95 dB SPL) were generated by conveying a short (0 01–0 1 msec) electrical pulse to a small earphone attached to an ear speculum which had been secured to the external ear of the animal

Solutions of 37 C were injected into the cochlea by inserting a small glass pipette (tip outer diameter less than 100 μ) into the hole previously drilled in the scala tympani The fluid injected traversed the length of

TABLE 1 *Composition and tonicities of solutions injected into the cochlea of guinea pigs.*

No.	Solution: Material	Solution: Dissolved in	Tonicity*	Number of experiments
1	Ringer's	—	1	8
2	Sucrose	Ringer's	1.5	5
3	Sucrose	Ringer's	2.0	5
4	Sucrose	Ringer's	3–6	5
5	Ringer's	—	0.75	7
6	Ringer's	—	0.5	4
7	Water	—	0	2

* An isotonic solution is assigned the tonicity of 1 so that hypertonic solutions are corresponding multiples of 1 and hypotonic solutions are decimal fractions.

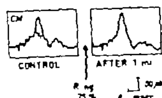


Fig. 1 The ffect of intracochlear i jection of slightly hypotonic sol tion on the cochlea potentials. E en though the intracochlea i jection of solution usually caused depression of the action potential (N) the i jection of solution of tonicity 0 75 (Ringer' solution 75%) caused a increase in N, amplitude N change i seen in the cochlear microphonic potential (CM)

In a previous paper (Feinmesser & Sohmer 1965) the injection *per se* caused a decrease in the cochlear action potential with no change (occasionally an increase in amplitude) in the cochlear microphonic potential A delay in action potential recovery (longer than the 10 minutes recovery period observed with injection of Ringer's solution alone) was therefore taken as a sign of deleterious effect of the solution on the action potentials. The results of this study are summarized in Table 2 It is seen that solutions ranging from isotonic to a hypertonicity of up to 2 had little or no effect on the cochlear potentials. This is indicated in the table by the short duration of the action potential depression and its rapid and complete recovery All solutions with tonicities of 2 or more caused a longer lasting (occa ionally complete) depression of the action potential amplitude with an occa ional increase in the amplitude of the cochlear microphonic potential. The duration of this depression and the duration of the recovery (if any) were dependent on the tonicity

The effect of hypotonic solutions on cochlear potentials

Several experiments were also performed with Ringer's solution diluted with distilled water The injection of Ringer s solution with a tonicity of 0 75 into the cochlea of 5 guinea pigs caused an initial increase in the mplitude of the action potential with no change in the amplitude of the cochlear microphonic potential (Fig. 1) The action potential returned to it pre-injection amplitude within about 10 minutes. Injection of Ringer's solution f tonicity 0.5 in 4 experiments caused an initial decrease in the amplitude of both the cochlear microphonic and action potentials which quickly (within 10 minutes) returned to their pre-injection amplitudes. The injection of distilled water caused a 100 drop in the amplitudes of both potential from which there was no apparent recovery

Cat (Round Window Application)

Th effect of hypotonic solution on cochlear potential

Hypotonic solutions applied to the round window caused a small increase in the amplitude of the action potentials with no change in the

that contact of the electrode with the liquid on the round window was often accompanied by an increase in amplitude of the cochlear potentials recorded probably due to an increase in conductivity at the point of electrode contact The indifferent electrode was a tached to the animal head holder The acoustic stimuli were generated and the cochlear potentials were recorded as described above

Table 2 shows the compositions and tonicities of the solutions applied to the round window of cats. These included various concentrations of sucrose choline chloride and sodium chloride in Ringer s solution or distilled water and several dilutions of Ringer s solution. About 0 03 ml of the solution at 37 C was applied to the round window and the effects on the cochlear potentials were observed

RESULTS

Guinea Pigs (Intracochlear Application)

The effect of hypertonic solutions on cochlear potentials

In 8 guinea pigs, the effects of intracochlear injections of increasing concentrations of sucrose in Ringer's solution were observed As mentioned

TABLE 3 *Effect of osmotic pressure of solutions listed in Table 1 on cochlear potentials in* guinea pigs

		Effect on AP[a]		Recovery of AP[b]		Effect on CM[c]	
No.	Tonicity	change	min	% recovery	min	change	min
1	1	↓ 71 (6)	3	100	11	↑ 56 (3)	2
		– (2)				– (4)	
2	1.5	↓ 100 (2)	4	100 (1)	16	↑ 1 (1)	3
				? (1)		– (4)	
3	2	↓ 92 (5)	43	– (3)		– (3)	
				100 (2)	33	↑ 27 (2)	2
4	3–6	↓ 100 (5)	∞	–		– (5)	
5	0 75	– (1)				– (7)	
		↓ 12 (1)	2				
		↑ 22 (5)	2	100	10		
6	0.5	↓ 87 (4)	3	100	10	↓ 31	9
7	0	↓ 100 (2)	∞	—		↓ 100	∞

– = no effect ↑ = a gmentation ↓ = depression, ? = effect not determined ∞ = no recovery

The number i pare theses represents th number f xperime ts with the a erage result cited

The effect n the a tion pot ntial (AP) i gi n the verag per ce t change b- served and the durati n f thi hange

[b] The recovery of the AP i gi n the a rag per cent recovery nd it tim dura ti n.

The effect n the cochlear microphoni pot nti l (CM) is gi en the verage pe cent change and the duration of this chang

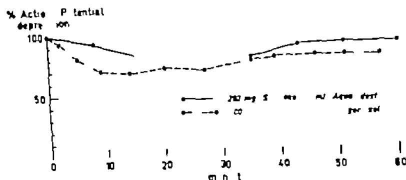


Fig. 2. Graphs showing the rates of action potential depression and recovery after round window application of two solutions of identical tonicities (2) but with different composition (200 mg sucrose/ml distilled water and 100 mg sucrose/ml Ringer solution). Note that the depression caused by the sucrose in Ringer's solution is greater and develops faster.

The effects of solutions of identical tonicities but with different ionic concentrations were compared. These solutions were prepared by dissolving the sucrose in distilled water or by dissolving a correspondingly smaller amount of sucrose in Ringer's solution. Although the effects were similar it generally appeared that the depression observed following application of the sucrose in Ringer's solution was greater and developed faster than that observed following the application of a sucrose solution of equivalent hypertonicity in distilled water. In most of the experiments partial recovery of the action potential was observed (Fig. 2).

In all of these cases, except for a small amplitude increase which occasionally accompanied the action potential depression, no change was observed in the amplitude of the cochlear microphonic potential. No depression of the microphonic potential was observed even upon application of a saturated sucrose solution (tonicity = 20) to the round window (Fig. 3). However, the application of a sodium chloride solution of equal tonicity (t = 20) caused a rapid decrease in the amplitudes of both the cochlear microphonic and action potentials.

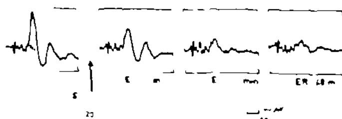


Fig. 3. The effect of round window application of a strongly hypertonic solution (tonicity 20) on cochlear potentials. The cochlear action potential (X) is strongly depressed, while the cochlear microphonic potential (CM) are unaffected.

TABLE 4 *Effect of osmotic pressure of solutions listed in Table 2 on cochlear potentials in cats*

No	Tonicity	Effect on AP[a] change	min	Recovery of AP[b] % recovery	min	Effect on CM[c] % change	min
1	< 1	↑ 17 (4)	18	—		—	
		– (1)					
2	1 (Ringer)	– (20)				—	
3	1 (Sucrose)	– (6)					
		↑ 18 (8)	24	—		—	
		↓ 30 (4)	37	– (2)		– (4)	
				89 (1)	26		
				? (1)			
4	2 (Sucrose in Ringer)	↓ 46 (5)	21	85	82	↑ 10	16
5	2 (Sucrose in water)	↓ 37 (8)	31	80	42	– (6)	
						↑ 5 (1)	3
						? (1)	
6	2 (Total)	↓ 39 (15)	27	83 (11)	64	↑ 15 (6)	14
				– (2)		– (6)	
				? (3)		? (3)	
7	3–5	↓ 37 (9)	27	89 (6)	54	↑ 10 (3)	10
				– (2)		– (4)	
				? (1)		? (2)	
8	> 5 (Sucrose)	↓ 72 (2)	44	– (1)			
				87 (1)	60	30 (1)	?
						– (1)	
9	> 5 (NaCl)	↓ 100 (1)	?	—		↓ 100	40

– = no effect ↑ = augmentation ↓ = depression, ? = not determined.

[a] Average per cent change which appeared and time to reach this change

[b] When there was recovery it is expressed as average per cent recovery and its time duration.

[c] Average per cent change which appeared and time to reach this change

cochlear microphonic potentials (4 experiments) Ringer's solution had no effect on either potential (Table 4)

The application of an *isotonic* sucrose solution (100 mg/ml distilled water) caused varied results a small increase in the action potential amplitude (8 experiments) an action potential amplitude decrease (in 4 other experiments) and in 3 animals, no change was observed (see Table 4) No change was observed in the amplitude of the cochlear microphonic potential

The effect of hypertonic solutions on the cochlear potentials

Higher sucrose concentrations (toxicity 2 or more) always caused depression of the cochlear action potential

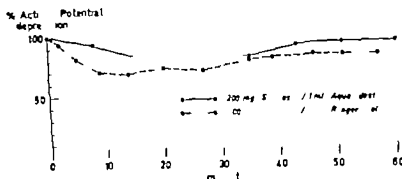


Fig. 2. Graphs showing the rates of action potential depression and recovery after round window application of t sol tions of identical tonicities (2) but with different compositions (200 mg sucrose/ml distilled water and 140 mg sucrose/ml Ringer's solution). Note that the depression caused by the sucrose in Ringer' solution is greater and develops faster.

The effects of solutions of identical tonicities but with different ionic concentrations were compared. These solutions were prepared by dissolving the sucrose in distilled water or by dissolving a correspondingly smaller amount of sucrose in Ringer's solution. Although the effects were similar it generally appeared that the depression observed following application of the sucrose in Ringer's solution was greater and developed faster than that observed following the application of a sucrose solution of equivalent hypertonicity in distilled water. In most of the experiments partial recovery of the action potential was observed (Fig. 2).

In all of these cases, except for a small amplitude increase which occasionally accompanied the action potential depression, no change was observed in the amplitude of the cochlear microphonic potential. No depression of the microphonic potential was observed even upon application of a saturated sucrose solution (tonicity = 20) to the round window (Fig. 3). However the application of a sodium chloride solution of equal tonicity (i.e. 20) caused a rapid decrease in the amplitudes of both the cochlear microphonic and action potentials.

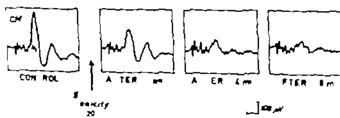


Fig. 3. The effect of round window application of strongly hypertonic solution (tonicity 20) on cochlear potentials. The cochlear action potential (N_1) is strongly depressed, while the cochlear microphonic potential (CM) are unaffected.

TABLE 4 *Effect of osmotic pressure of solutions listed in Table 2 on cochlear potentials in* cats

		Effect on AP[a]		Recovery of AP[b]		Effect on CM[c]	
No.	Tonicity	% change	min	% recovery	min	% change	min
1	<1	↑ 17 (1)	18	—		—	
		— (1)					
2	1 (Ringer)	— (20)				—	
3	1 (Sucrose)	— (6)					
		↑ 18 (8)	21	—		—	
		↓ 36 (1)	37	— (2)		— (4)	
				89 (1)	26		
				? (1)			
4	2 (Sucrose in Ringer)	↓ 48 (5)	21	85	82	↑ 10	16
5	2 (Sucrose in water)	↓ 37 (8)	31	80	42	— (6)	
						↑ 5 (1)	3
						? (1)	
6	2 (Total)	↓ 39 (15)	27	83 (11)	64	↑ 15 (6)	14
				— (2)		— (6)	
				? (3)		? (3)	
7	3–5	↓ 37 (9)	27	89 (6)	54	↑ 10 (3)	16
				— (2)		— (4)	
				? (1)		? (2)	
8	>5 (Sucrose)	↓ 72 (2)	44	— (1)			
				87 (1)	60	↑ 30 (1)	?
						— (1)	
9	>5 (NaCl)	↓ 100 (1)	?	—		↓ 100	40

— = no effect ↑ = augmentation ↓ = depression ? = not determined.

[a] Average per cent change which appeared and time to reach this change.

[b] When there was recovery it is expressed as average per cent recovery and its time duration.

[c] Average per cent change which appeared and time to reach this change.

cochlear microphonic potentials (4 experiments) Ringer's solution had no effect on either potential (Table 4)

The application of an *isotonic* sucrose solution (100 mg/ml distilled water) caused varied results a small increase in the action potential amplitude (8 experiments) an action potential amplitude decrease (in 4 other experiments) and in 3 animals, no change was observed (see Table 4) No change was observed in the amplitude of the cochlear microphonic potential

The effect of hypertonic solutions on the cochlear potentials

Higher sucrose concentrations (toxicity 2 or more) always caused depression of the cochlear action potential

frog nerve fibers observed during application of hypotonic solutions and a mechanism, similar to that advanced by Lorente de No (1947) may be responsible

Cochlear Microphonic Potentials

The absence of a depressant effect of *hypertonic* solutions on the *cochlear microphonic potential* is unexpected for it seems to indicate either that the anatomical structures which contain the generation apparatus of the cochlear microphonic potential are impermeable to water or solutes (so that hyperosmotic conditions are not reached within them) or that the generation apparatus is not affected by the osmotic pressure of its surroundings. A definite conclusion as to which is responsible for this phenomenon is complicated by the fact that there is still no definite proof as to the structures and mechanisms responsible for generation of the cochlear microphonic potential.

The application of hypertonic solutions occasionally caused an augmentation of the cochlear microphonic potentials in addition to depression of the action potential. A similar cochlear microphonic potential augmentation has also been observed to accompany the cochlear action potential depression caused by streptomycin (Feinmesser & Sohmer 1965) and by electrical stimulation of the contralateral olivo-cochlear bundle (Sohmer 1965). An attempt has been made to explain this phenomenon as being due to an intracochlear resistance change and not to any increase in cochlear microphonic potential generation (Sohmer 1965)

ZUSAMMENFASSUNG

Bei Versuchen mit Medikation der Ohrschnecke muss der osmotische Druck der zu verwendenden Lösungen in Betracht gezogen werden. Innerschneckeninjektion bei Meerschweinchen und Schneckenfensterbehandlung bei Katzen mit Lösungen von doppeltem osmotischem Druck der üblichen Ringerschen Lösung ergaben Senkung des Aktionspotentials. Lösungen mit bis zu zwanzigfachem Druck der üblichen Ringerschen Lösung hatten keine Wirkung auf das Mikrophonpotential der Schnecke. Es wurde versucht, diese Ergebnisse zu analysieren.

REFERENCES

Aldred, P., Hallpike, C. S. and Ledoux, A., 1940. Observations on the osmotic pressure of the endolymph. *J Physiol* 98, 446.

Cutt, R. A., 1963. The effect of the local application of middle ear drugs upon the cochlear potentials. *Laryngoscope* 73, 702.

Feinmesser, M. and Sohmer, H., 1965. Influence of streptomycin and dihydrostreptomycin on the cochlear potentials of the guinea pig. *Ann. Otol.*, 74, 48.

Hennebert, D. and Fernandez, C., 1959. Ototoxicity of quinine in experimental animals. *Arch Otolaryng (Chic.)* 70, 321.

Koide, Y., Hata, A., and Hamano, R., 1966. Vulnerability of the organ of Corti in poisoning. *Acta Otolaryng (Stockh.)* 61, 332.

In 2 experiments, choline chloride solutions were tested In both, an isotonic solution (23 mg/ml distilled water) caused a small action potential augmentation while a *hypertonic solution with a tonicity of 2* caused a depression in the amplitude of the action potential which showed signs of recovery with time In neither experiment were there any changes in the amplitude of the cochlear microphonic potential

DISCUSSION

Hypertonic Solutions

This study has thus *delineated* the *upper concentration range* which should be observed in the study of ototoxic drug effects by means of local application In *guinea pigs* concentrations approaching a tonicity of twice that of ordinary Ringer's solution are capable of causing depression of the action potential Tonicities of up to 6 times that of Ringer's solution, however did not have any effect on the cochlear microphonic potential In *cats* isotonic sucrose solutions occasionally caused depression of the action potential probably because of diffusion of sucrose molecules through *the round window membrane* leading to hypertonicity within the cochlea. Solutions with a tonicity twice that of Ringer's solution always caused action potential depression Highly concentrated solutions with tonicities of up to 20 times that of Ringer's had no effect on the cochlear microphonic potential

In experiments involving cochlear application of drugs suspected of being ototoxic in concentrations approaching those outlined above it would seem advisable, therefore to first apply a sucrose solution the tonicity of which is *equal to that of the drug solution to be used. It must* be added however that the results of cochlear application of drugs may differ from the results of systemic injection due to the different routes to the cochlea in each with different membrane barriers Such differences have been reported with respect to streptomycin (Feinmesser & Sohmer 1965)

An attempt to explain the mechanism by which hyperosmotic solutions depress the action potential response of the cochlea is made difficult by *the fact that the fluid physiology of the cochlea is not sufficiently known* There is no agreement as to the permeability of the cochlear membranes (Tonndorf Duval & Reneau, 1962 Schuknecht & Seifl, 1963) and as to the location of formation and reabsorption of the cochlear fluids (Vosteen 1961) In addition there may be relative differences in hydrostatic or osmotic pressures between the various scalae (Weille *et al.*, 1961 Lawrence 1965 Alfred *et al.*, 1940)

Hypotonic Solutions

The increase in the amplitude of the action potential during application of slightly *hypotonic* solutions is similar to the increase in spike height in

Ad oto-laryngologica 64, 63–71 (1967)

OESTRIOL SUCCINATE

Its Effect on the Haemorrhage in Adenotonsillectomy and Tonsillectomy

O TRUE PEDERSEN and P H NIELSEN
Copenhagen Denmark

From the D partm nt of Otolaryngology (Head M Rottwitt Schmidt) Frederiksb rg Hospital Copenhagen

I order to assess the haemostati effect of oestroge s, the amount of haemorrhage in de oton illectomy and ton illectomy was measured nd th number f post perativ haemorrh ges analysed in two groups f patients. One group received Oestriol Succinat while the other group received placebo. The total average haemorrh ge was in the same range of magnitude in both groups. I th group treated with Oestri l Succinat there was smaller number f post perative haemorrhages that did not require treatm nt (lot) and the adenotonsillect mized pat ents had less operati e haemorrhage in relation to a corresponding n mber f the untreated patients. Thi appears to indicate an effect of th substance on capillary bleedings.

F r many years oestrogenic preparations have been studied for their haemostatic effect In 1941 Jacobson submitted a study on a form of epistaxis which he believed was due to a fall in the blood level of oestrogen. Since that time numerous authors have tudied the haemostatic effect of oestrogens, experimentally as well as within clinical medicine and surgery

Johnson (195 1959) and Owren (1957) f und that the effect was due t alteration in the coagulation mechanism of the blood, the plasma level f AC globulin and prothrombin increasing, while the content of anti thrombin f ll fter intravenous injection of Premarin which is a substance with oestrogenic ffect extracted from the urine f pregnant mares.

Schiff & Burn (1961) did not find these alterations in the coagulation mechanism f the blood. Nevertheless, they felt there wa a definite haemostatic effect, due to an action upon the ground substance in and around the walls of the small vessels facilitating th healing of any lesions. Theref re, the effect was supposed to be most marked upon capillary bleeding These findings were confirmed by Polliwoda (1962)

Withers (1960) in tonsillect mies upon 400 patients, 200 of whom received Premarin preoperatively f und no difference in the severity of operative bleeding between the two groups. He did not state how he measured the blood loss. During the first 24 hours after the operation

LAWRENCE, M 1965: Fluid Balance in the inner ear Ann Otol 74 486
LORENTE DE NO, R 1947 A study of nerve physi logy Studies fr m the Rockefell r Institut for Medical Research Vol 131
RAHM W E., JR., STROTHER, W F., GULICK, W L., and CRUMP J F 1959 The effects of topical anesthetics upon the ear Ann Otol 68 1037
SCHUKNECHT H F and SEIFI, A E., 1963 Experim ntal observations on the fluid physiology of the inner ear Ann Otol 72 687
SOHMER, H 1965 The effect of contralateral olivo-cochlea bundle stimulation on the cochlear potential evoked by acoustic stimuli of various frequen ies and i ten ities. Acta Ot laryng (Stockh.) 60 59
TASAKI, I and FERNÁNDEZ, C., 1952 Modification of cochlea microphonics and action potentials by KCl solution and by direct currents. J Neurophysi l 15 407
TONNDORF J DUVAL, A J and REneau J P., 1962 Permeability of intr cochle r membranes to variou vital stains. Ann Otol 71 801
VOSTEEN K H 1961: Neue Aspekte zu Biologie und Pathologie die Innenohres. Arch. Ohrenheilk 178 1
WEILLE, F L., O BRIEN H F RAHN, P A DERSO A CLARK, L., JAKO, G nd IRWIN, J W 1961 Pressures of the labyrinthine fluids. II Ann Ot l 70 525.
WEVER, E. G and LAWRENCE, M 1954 Physi logical Acoustics Princeton University Press, Princeton

Rog ff Lab of Physiology
The Hebrew University—
Hadassah Medical School
Jerusalem I rael

Rec ived September 8 1966

OESTRIOL SUCCINATE

Its Effect on the Haemorrhage in Adenotonsillectomy and Tonsillectomy

O. TRUE PEDERSEN and P. H. NIELSEN
Copenhagen, Denmark

From the Department of Otolaryngology (Head: M. Rottwitt Schmidt) Frederiksberg Hospital, Copenhagen

In order t assess the ha mostatic effect f oestrogens, the amount of haemorrhage in adenot sillectomy nd tonsillectomy was measured nd the number f postoperati haemorrhages nalysed in two groups of patients. On group received Oestriol Succinat whil the other group received placebo. Th t tal verage haemorrhage was in the same ra ge of magnitud in both groups. In the group treated with Oestriol Succinat there w smaller number of postoperative haemorrhages that did not require treatment (clot) and the denotonsillectomized pati t had less perati e haemorrhage in relation to a corresponding number of the untreated patients. Thi appears to indicate an effect of the substance on cap llary bleedings.

For many years oestrogenic preparations ha e been studied for their haemostatic effect. In 1941 Jacobson submitted a study on a form of epistaxis which he believed was due to a fall in the blood level of oestrogen. Since that time numerous auth rs ha e studied the haemostatic effect of oestrogens, xperimentally as well s within clinical medicine and surgery.

Johnson (1957, 1960) and Owren (1957) found that the effect was due to alteration in the coagulation mechanism of the blood, the plasma level of AC globulin and prothrombin increa ing, while the content of anti thrombin fell after intravenous injecti n of Premarin which is a substance with oestrogenic effect extracted from the urine of pregnant mares.

Schiff & Burn (1961) did not find these alterations in the coagulation mechanism of the blood. Nevertheless, they felt there was a definite haemostatic effect, due to n action upon the ground substance in and around the walls of the small vessels facilitating the healing of any lesions. Therefore, the effect was supposed to be m st marked upon capillary bleeding. These findings were confirmed by Polliwoda (1962).

Wilbers (1960) in tonsillectomies upon 400 patients, 200 of whom recei ed Premarin preoperatively found no difference in the severity of operative bleeding between the two groups. He did not state how he measured the blood loss. During the fir t 24 h urs after the operati n

there was a decrease in the rate of postoperative haemorrhage of from 2.5% to 1% in the group that had been treated preoperatively In 34 tonsillectomized patients Jacobson (1954) observed a definite decrease in the incidence of postoperative haemorrhage In a series of 140 tonsillectomized patients, half of whom had received Oestriol Succinate Zechner (1964) found a distinctly reduced operative bleeding However the quantity of the blood loss was merely estimated and this greatly reduces the value of this observation In secondary haemorrhages following adenotomy in 10 patients, Menger (1955) obtained rapid haemostasis by the use of intramuscular oestrogen

In Scandinavia, the effect of oestrogens has been studied *inter alia* by Nilsson (1960) and by Borchgrevink *et al* (1960) and neither team found any definite haemostatic effect of Premarin *in vitro* or *in vivo* Studying the effect of Premarin on 24 patients with epistaxis and two tonsillectomized patients, *Hall & Ursin Holm (1959) found no definite effect However* they pointed out the need of placebo experiments and studies in which control series were run

Since there have not been any major systematic analyses of the haemostatic effect of oestrogens in tonsillectomy and since previous authors have not agreed in respect to the effect we tried to assess the effect of one of these preparations upon the amount of haemorrhage in adenotonsillectomy and tonsillectqmy and to ascertain whether the substance influenced the incidence of postoperative haemorrhage

The preparation used in the present study was Oestriol Succinate the non proprietary name of the water soluble sodium salt of oestriol 16-17 disuccinate [1]

METHOD AND MATERIAL

The investigation was carried out as an experiment using two preparations coded by the manufacturers One contained the active substance in solution the other one only the solvent No person involved in the experiment knew beforehand which preparation contained the active substance On even dates, the treatment was started with the preparation labelled A, on odd dates with the preparation labelled B After the expiry of the experimental period and after the experimental results had been analysed it was reported *by the manufacturers that group A had received Oestriol* Succinate, while group B had received the placebo preparation The preparation was administered by the intramuscular route, except for the injection just prior to the operation which was, for practical reasons, intravenous Every day for 7 consecutive days, all patients under 8 years of age received 10 mg and patients over 8 years 20 mg per injection The first injection was administered on the eve of the operation No patient under 6 years of age was included in the trial

[1] Ki dly supplied by N V Orga n, Oss, H lland Trad n me: stypian n.

Table 1 *Case material by number average age and sex*

Group	No. of patients	Average age	Males	Females
A	40	16.8	17	23
B	39	15.0	14	25

Table 2 *Case material by sex and age*

Age	Group A			Group B			A + B
	Males	Females	Total	Males	Females	Total	Total
6–10	2	2	4	1	2	3	7
11–15	3	4	7	1	8	9	16
16–20	4	12	16	7	11	18	34
21–25	6	2	8	2	3	5	13
26–30	0	2	2	0	0	0	2
31–35	2	0	2	1	1	2	4
36–40	0	0	0	1	1	2	2
41–45	0	0	0	0	0	0	0
46–50	0	1	1	0	0	0	1

In all cases the surgical procedure consisted in tonsillectomy or adenotonsillectomy in the dorsal recumbent position under general anaesthesia. The operations were performed by four different surgeons.

The amount of haemorrhage during the operations was measured, partly as the quantity collected by suction, measured in ml, and partly as the quantity absorbed by the packs, measured in g. At the completion of the operations the packs were weighed, and from the result we subtracted the weight of a corresponding number of dry packs. These values together were used to denote the haemorrhage during the operations, no regard being paid to the minor inaccuracy caused by adding the figures in ml and in g.

The material comprised 79 patients whose distribution by number average age and sex is shown in Table 1 while Table 2 gives the age distribution.

In group A 10 patients had adenotonsillectomy average age 11 1 years. 30 patients had tonsillectomy. None of the patients had previously been tonsillectomized.

Group B comprises 10 patients who had adenotonsillectomy average age 12.4 years, and 27 patients who had tonsillectomy. Two patients had previously undergone tonsillectomy (Table 3).

Out of regard for a possibly increased bleeding tendency in patients with acute tonsillar changes, we analysed the number of patients who had had

TABLE 3 *Distribution of patients by type of operation and number of patients with acute tonsillar changes in the various groups*

Group	Adenotonsillect.	Tonsillect.	Re-tonsillect	Patients with acute tonsillar changes
A	10	30	0	6
B	10	27	2	7

acute tonsillitis immediately prior to admission and who still showed objective changes of the tonsillar mucosa at the time of the operation This was found in 6 patients of group A and 7 patients of group B None of these patients had elevation of temperature at admission or during the post operative course Only one patient had the operation under penicillin cover"

RESULTS

The amount of the average haemorrhage in the various groups is given in Table 4 The maximum and minimum blood loss was 590 and 59 None of the operated patients had blood transfusions In two patients of both groups packs were sutured to the tonsillar beds because of severe diffuse bleeding which yield to the usual treatment (ligation suturing or electrocoagulation) Table 5 gives the number of patients with postoperative haemorrhage A distinction is made between haemorrhage which required and haemorrhage which did not require treatment manifesting itself only in the form of clots in the tonsillar beds

The total average haemorrhage in patients with acute tonsillar changes was somewhat in excess of the average for the material as a whole However this was to be expected

The anaesthetics did not appear to have any influence upon the amount of haemorrhage The haemorrhage in the various age groups was of approximately the same quantity In respect to the quantity of the bleeding

TABLE 4 *Average haemorrhage in all patients and in the group of adenotonsillectomized patients*

Group	Average haemorrhage in all patients	Average haemorrhage in adenotonsillectomized patients
A	228	161
B	226	236

TABLE 5 *Number of patients with postoperative haemorrhage*

Group	Haemorrhage requiring treatment: Primary	Haemorrhage requiring treatment: Secondary	Haemorrhage that did not require treatment
A	2	0	4
B	0	2	7

and the incidence of postoperative haemorrhage, there was no decisive difference between the various surgeons.

There were no other operative or postoperative complications of any kind

DISCUSSION

The patients of this material were not selected, but represent all patients over 6 years of age who were admitted for adenotonsillectomy or tonsillectomy during the experimental period, for primary operation or re-operation. A lower age limit was fixed because of the discomfort connected with the parenteral administration of the preparation, but no upper age limit was fixed. Neither the history nor supplementary tests, when done revealed an increase in the bleeding tendency in any of the patients. To secure experimental conditions as uniform as possible parenteral administration was preferred

In respect to the assessment of the amount of haemorrhage, the blood collected by suction of course contains some saliva and other secretions for which, in fact, the values ought to be corrected. However the result may be used uncorrected as a relative measure under otherwise uniform experimental conditions. Thus, although the values measured do not represent the exact blood loss, they ought to make up relative quantities which are directly comparable. We feel that this method is fully justified in the present experimental series and preferred it to other more difficult methods for measuring the blood loss.

Provided that the above mentioned theories on the actions of the preparation are valid, the effect would be expected to manifest itself in the capillary haemorrhage during the operation as well as during the post operative period. It must be consistent therefore not only to administer the drug preoperatively but also postoperatively to assess its effect

In the present material there was no major difference in the total, average haemorrhage between groups A and B. On the other hand, there was a distinct difference between the adenotonsillectomized patients of the respective groups, the values being 154 and 236 respectively. However each group comprised only 10 patients, so that the results can only be accepted

TABLE 3 *Distribution of patients by type of operation and number of patients with acute tonsillar changes in the various groups*

Group	Adenotonsillect.	Tonsillect	Re-tonsillect.	Patients with acute tonsillar changes
A	10	30	0	6
B	10	27	2	7

acute tonsillitis immediately prior to admission and who still showed objective changes of the tonsillar mucosa at the time of the operation This was found in 6 patients of group A and 7 patients of group B None of these patients had elevation of temperature at admission or during the post operative course Only one patient had the operation under penicillin cover"

RESULTS

The amount of the average haemorrhage in the various groups is given in Table 4 The maximum and minimum blood loss was 590 and 59 None *of the operated patients had blood transfusions* In two patients of both groups packs were sutured to the tonsillar beds because of severe diffuse bleeding which yield to the usual treatment *(ligation* suturing, or electrocoagulation) Table 5 gives the number of patients with postoperative haemorrhage A distinction is made between haemorrhage which required and haemorrhage which did not require treatment manifesting itself only in the form of clots in the tonsillar beds

The total average haemorrhage in patients with acute tonsillar changes was somewhat in excess of the average for the material as a whole However this was to be expected

The anaesthetics did not appear to have any influence upon the amount of haemorrhage The haemorrhage in the various age groups was of approximately the same quantity In respect to the quantity of the bleeding

TABLE 4 *Average haemorrhage in all patients and in the group of adenotonsillectomized patients*

Group	Average haemorrhag in all patients	Average haemorrhage in adenotonsillect mized patient
A	228	154
B	226	236

Schiff, M., and Burn, H. F. 1961 The effect of intravenous estrogens on ground substance. *Arch. Otolaryng* (Chic.) 73, 43.

Whitman, B. T. 1960: Facility i tonsillo-adenoidectomy management. *Arch. Otolaryng* (Chic.) 72 183.

Zechner, G. 1964 Zur Blutungsprophylaxe bei Tonsillektomie und Adenotomie. *Mschr Ohrenheilk.*, 98, 178.

Dept. of Otolaryngology
Frederiksberg Hospital
Copenhagen, Denmark

Received November 10 1964

with some reserve The total incidence of postoperative haemorrhage was 5% in both groups

Haemorrhage requiring treatment occurred in two patients of each group, while haemorrhage which did not require treatment occurred in two patients of the oestrogen treated group and in five patients of the placebo group

It should be mentioned that postoperative haemorrhage from the rhinopharynx did not occur in any case

The use of the preparation did not give rise to any side effects This is in accordance with the findings of others (Polliwoda Withers, Jacobson, Zechner)

Summing up it may be said that the effect of Oestriol Succinate manifested itself in a less marked blood loss in the adenotonsillectomized patients and in a smaller number of postoperative haemorrhages requiring treatment

ZUSAMMENFASSUNG

Zur Beurteilung der hämostatischen Wirkung östrogener Stoffe hat man die Grösse der Blutung durch Adenotonsillektomie und durch Tonsillektomie gemessen sowie die Anzahl der Nachblutungen bei 2 Gruppen Patienten aufgestellt Hiervon bekam die eine Gruppe Östriolsuccinat während die andere Gruppe ein entsprechendes Suggestionsmittel erhielt Die gesamte durchschnittliche Blutung war in beiden Gruppen von derselben Grösse In der mit Östriolsuccinat behandelten Gruppe wurde eine kleinere Zahl von Nachblutungen (Koagulierungen) gefunden die keine Behandlung erforderte und die mit Adenotonsillektomie behandelten Patienten hatten eine kleinere operative Blutung im Verhältnis zu der entsprechenden Anzahl Patienten der nicht behandelten Gruppe Dies sollte als Ausdruck für eine Wirkung des Stoffes bei Kapillarblutungen anzusehen sein.

REFERENCES

BORCHGREVINK, C. F ANDERSEN, R HALL, J H TTELA D, K nd L RSEN HOLM A 1960 Premarin a a ha most ti gent *Brit Med J* 2 1645

HALL, J G nd LARSEN HOLM, A., 1959: Epistax behandlet med oestrogenpræparat *T No k Lægef r n* 79 58

J COBSO I 1954 Spont eou hem rrhag *Arch Otolaryng* (Chi) 59 523

JOHNSON, J F 1957 Cha ge in pla ma prothrombin, Ac-globulin nd a tithrombi co centration foll wi g intra n dmi istration of estroge *Proc Soc Exp Bi l Med* 94 92.

— 1950: Intra nou estrog ns nd cert in f ct rs in blood coagul ti n *Clin Med* 6 637

MENGER, H C., 1955 Estrogen giv parent rally to control epist l a d hem rrhag after d noidectomy *JAMA* 159 546

NILSSO I M 1960 Premarit intra nb som h m statikum. N *rd Med* 64 1185

OWREN P A 1957: Coagulati n f blood. N *rthwest Med* 56 31

POLLIWODA, H 1962 Estrog ns and hemorrhag *JAMA* 182 815

Personen untersuchte man ein Ohr bei 10 Personen beide Ohren. Insgesamt wurden also 90 Ohren geprüft.

Die Untersuchungen umfassten 4 Gruppen

gesunde Personen mit normalem Gehör
Personen mit Schalleitungsschwerhörigkeit,
Personen mit Perzeptionsschwerhörigkeit,
Personen mit gemischter Schwerhörigkeit.

Die Untersuchungen wurden in der Camera silens der Otolaryngologischen Klinik in Szczecin an Hand des Audiometers der Firma Pedersen, zu welchem eine Einrichtung angebaut wurde die das Entstehen kurzer Töne von der Zeitdauer 0,001–1 sek ermöglichte, durchgeführt. Das Ein und Ausschalten des Tones erfolgte ohne Knakken in dem Moment, in welchem die Sinusoide der Spannung des Wechselstromes, der im akustischen Generator entsteht, durch den Nullwert tritt.

Es wurde die Hörschwelle für den Dauerton und Töne von der Zeitdauer von 1 0,5, 0,2, 0 1 0,05, 0 02, 0 01 0 005, 0 002 und 0,001 sek fest gestellt. Die Zeitdauer des Tones wurde so gewählt, dass sie in sich die vollkommene Zahl der Schwingungsperioden des gegebenen Tones enthielt und deshalb wurden bei der Frequenz 250 Hz die Zeitdauer von 0 008 und 0,004 sek statt 0 01 und 0,005 sek verwendet Die Schwellenintensität wurde mit einer Genauigkeit bis zu 1 db festgestellt.

Die Abhängigkeit zwischen der Schwellenintensität und der Zeitdauer der Töne wurde graphisch dargestellt. Auf der Ordinate haben wir in der logarithmischen Decibel Skala die Intensitäten der Töne aufgetragen, auf der Abszisse — auch in der logarithmischen Skala — die Zeit in Sekunden Für jede Frequenz erhielten wir so eine Kurve der Schwellenintensitäten der Töne von verschiedener Zeitdauer

ERGEBNISSE

Personen mit normalem Gehör

Bei jeder von den 25 gesunden Personen mit normalem Gehör wurde ein Ohr untersucht. Die Untersuchung wurde mit Tönen in der Frequenz 100 250, 1000 4000 und 10 000 Hz durchgeführt. Die Mittelwerte der Schwellenintensitäten dieser Personengruppe für die Töne von verschiedener Frequenz und Zeitdauer wurden in der Abb. 1 dargestellt.

Die Hörschwelle für den Ton von der Zeitdauer 1 sek befindet sich tiefer als die Schwelle für den Dauerton. Dieser Unterschied hängt von der Adaptation ab und beträgt bei der Frequenz von 100 Hz 10 db, bei 250 Hz 8 db, bei 1000 Hz 9 db, bei 4000 Hz 7 db und bei 10 000 Hz 17 db.

Die Abkürzung der Zeitdauer des Tones von 1 sek bis zu 0,5 sek hat keinen Einfluss auf den Wert der Schwellenintensität, jedoch bei der Abkürzung des Tones unter 0,5 sek wächst die Schwellenintensität. Die Per

Acta oto-laryngologica 64 72–80 (1967)

DIE HÖREMPFINDLICHKEIT FÜR KURZDAUERNDE TÖNE

M Taniewski
S c ecin Polen

Aus der Otolaryngologischen Klinik der Medi inischen Akademie (Direktor Prof J Taniewski) S c ecin

An Hand von audiometrischen Untersuchungen mit kurzdauernden Tönen wurde festgestellt dass bei gesunden Personen die Hörschwelle bei der Abkürzung der Zeitdauer des Tones unter 0,5 sek wächst. Der Unterschied der Schwellenintensität des 1 sek dauernden und 0 001 sek dauernden Tones beträgt ungefähr 50 db Bei der Frequenz von 1000 Hz ist das Produkt des Schwellendruckes eines Tones durch seine Zeit dauer ein konstanter Wert Der Hörverlust für einen Dauerton wird immer von dem Hörverlust für kurzdauernde Töne begleitet Bei der Schalleitungsschwerhörigkeit vom Dampfungstyp sowie in Fällen der Beschädigung des Ganglions oder des N *Cochlearis* war die Verminderung der Hörempfindlichkeit von der Zeitdauer des Tones unabhängig Bei der Schwerhörigkeit vom Typ der elastischen Versteifung bei der Frequenz von 1000 Hz war der Hörverlust kleiner für Töne von kürzerer Zeitdauer Jedoch bei der Frequenz von 250 und 4000 Hz war er *genauso für Töne von kürzerer und längerer Zeitdauer* In Fällen der Schallempfindungsstörung vom Typ der Hörzellenbeschädigung trat für Töne von kürzerer Zeitdauer eine relative Besserung des Gehörs auf

Noch bis vor kurzem nahm man an dass das menschliche Ohr auf Tone von langer und kurzer Zeitdauer genauso reagiert Letztens stellte man jedoch fest, dass wenn sich die Zeitdauer des Tones unter einem gewissen Wert verkurzt seine Schwellenintensität wächst (Paiva, 1956 Langenbeck 1959 Miskolczy Fodor 1953)

Nach Kietz (1961) hängt der Unterschied zwischen der Schwellenintensität der Tone von längerer und kürzerer Zeitdauer nicht von der Frequenz des Tones ab nach Caspers, Lerche & Plath (1960) Makarow & Goldburt (1962) jedoch ist die Abhängigkeit der Schwellenintensitat von der Zeitdauer des Tones verschieden für Töne von verschiedenen Frequenzen Bis jetzt ist es noch nicht geklart wie schwerhörige Menschen kurzdauernde Tone empfangen

UNTERSUCHUNGSMATERIAL UND METHODE

Es wurden die Hörschwellenintensitäten für kurzdauernde Töne bei 80 Personen mit normalem und beeinträchtigtem Gehör untersucht Bei 70

b

Abb. 2. Die Schwellenintensität für kurzdauernde Töne bei Schalleitungsschwerhörigkeit. *a* Dämpfungstyp; *b* elastische Versteifung; ○ 250 Hz; ●, 1000 Hz; 4000 Hz; —— die Standardabweichung der normalen Werte; *D* Dauer.

In 12 Fällen haben wir die Schalleitungsschwerhörigkeit vom Dämpfungstyp festgestellt. Die Mittelwerte ihrer Hörschwellenintensitäten für die Töne von verschiedener Zeitdauer wurden in der Abb. 2 *a* dargestellt.

Bei der Frequenz von 250 Hz betrug der Unterschied zwischen den Schwellenintensitäten der Töne von 1 sek und 0,004 sek Dauer in dieser Gruppe 39 db. Zwischen den Schwellen der Töne von der Zeitdauer 1 sek und 0 001 sek betrug der Unterschied bei 1000 Hz Frequenz 55 db und bei 4000 Hz 45 db.

In 11 Fällen wurde Schalleitungsschwerhörigkeit vom Typ der elastischen Versteifung diagnostiziert. Die Mittelwerte der Schwellenintensitäten dieser Gruppe sind in der Abb. 2 b dargestellt. Der Unterschied zwischen den Schwellen der Töne von 1 sek und 0 004 sek Dauer betrug hier bei 250 Hz 38 db, bei 1000 Hz und Zeitdauer von 1 sek und 0 001 sek 27 db und bei 4000 Hz und Zeitdauer von 1 sek und 0 001 sek 45 db

Die Kranken mit einer Schallempfindungsstörung wurden an Hand der

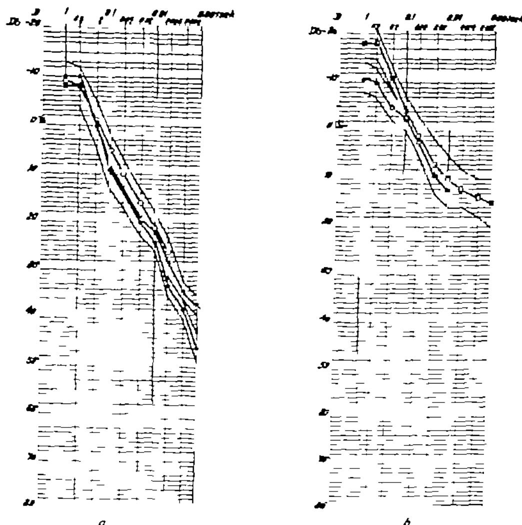


ABB. 1 Di Abhängigkeit der Schwellenint nsität vo der Zeitdau r des Tones bei normalhörenden P rs en. Mitt lwerte fü △, 100 Hz ○ 250 Hz ●, 1000 Hz 4000 H □, 10 000 Hz —— di Standardabweichung d Mitt lwert D Dauert n

zeptionsschwelle des Tones von 0 1–0 2 sek Dauer ist dur Schwelle des Dauertons gleich

Bei der Frequenz von 250 Hz betragt der Unterschied zwischen den Schwellenintensitäten der Töne von 1 sek und 0 004 sek Dauer 35 db, bei 1000 Hz ist der Unterschied der Schwellenintensitäten der Töne von 1 sek und 0 001 sek 56 db gleich und bei 4000 Hz beträgt dieser Unterschied für die Töne von 1 sek und 0 001 sek 45 db

Die Standardabweichung der Messungen betrug 2–5 db

Schwerhörige Personen

Bei schwerhörigen Personen untersuchte man die Schwelle der Perzeption für kurzdauernde Töne von der Frequenz 250 1000 und 4000 Hz.

Die Schalleitungsschwerhorigkeit haben wir nach der Klassifikation von Langenbeck in 2 Typen geteilt in den Typ der elastischen Versteifung und den Dampfungstyp

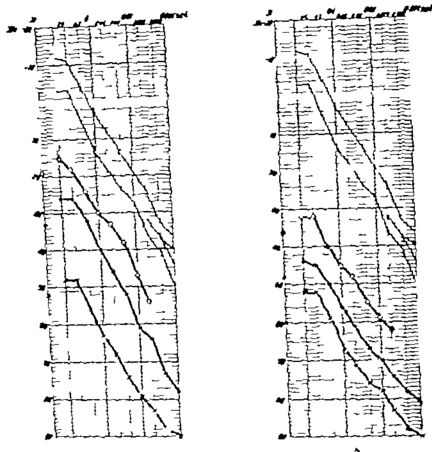
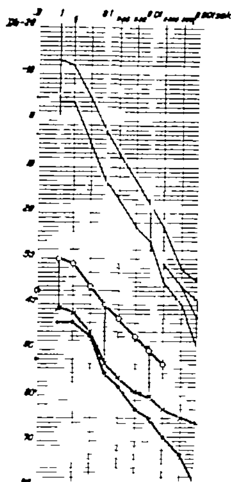


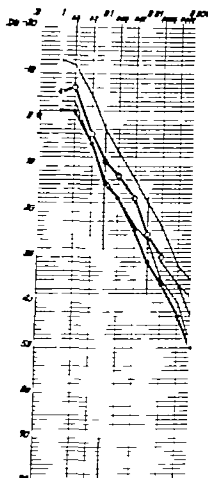
Abb. 4 Die Schwellenintensität für kurzdauernde Töne *a*, Schallempfindungsstörung vom Typ der Beschädigung des Ganglion oder des N. Cochlearis *b* Schwerhörigkeit gemischte T *p* O, 250 H, 1000 H; 4000 H; ——, di Standardabweichung der normalen Werte D D werten.

Bei der Frequenz von 250 Hz beträgt hier der Unterschied zwischen den Schwellenintensität n der Töne von 1 sek und 0,004 sek Dauer 23 db und der Unterschied zwischen den Schwellen der Töne die 1 sek und 0 001 sek dauern, beträgt bei der Frequenz on 1000 Hz 33 db und bei der Frequenz n 4000 Hz 25 db.

Die Mittelwerte der Hörschwellenint nsitäten für kurzdauernde Töne von der Frequenz 250 und 1000 Hz dieser Fälle in welchen das Gehör für Dauertöne dieser Frequenzen normal war haben wir in Abb. 3 b dargestellt. In der Frequ nz 250 Hz betrug der Unterschied der Schwellenintensitäten zwischen 1 sek und 0,004 sek dauernden Töne in diesen Fällen 30 db und in der Frequenz von 1000 Hz ergab sich ein Unterschied zwischen den Tönen von 1 sek und 0 001 sek Dauer 2 db.

In 10 Fällen stellten wir eine Schallempfindungsstörung vom Typus der





a *b*

A 3 Die Schw ll ni t nsit ten für kurzdauernde Töne bei Schallempfi dungs tö ung vom Typ de Hörz llenbeschädigung *a* Falle in w lcl e da G hö f Da ert ne Le ht iligt wa *b* Fälle in w l hen d s G hö fü D u rto norm l war O 250 H ● 1000 Hz 4000 Hz —— di St nd rd bw i hung de normal W rte *D* D u rt n

überschwelligen audiometrischen Untersuchungen in 2 Gruppen eingeteilt Zur ersten Gruppe zählten wir die Fälle bei welchen eine Beschädigung der Hörzellen im Cortischen Apparat vorhanden war zur zweiten dagegen diejenigen bei welchen eine Beschädigung des Ganglions oder des N Cochlearis existierte

Eine Gehorstörung vom Typus der Hörzellenbeschädigung wurde in 19 Fällen festgestellt Manche von diesen Kranken litten nur an Perzeptionsbenachteiligung für hohe Töne bei anderen dagegen wurde das Gehör im Bereich aller Töne gestort So wurde die Schwerhörigkeit fur den Dauerton von 250 Hz in 5 Fällen für den 1000 Hz Ton in 13 Fallen und für den 4000 Hz Ton in 19 Fällen festgestellt

In der Abb 3 *a* wurden die Mittelwerte der Schwellenintensitäten für kurzdauernde Tone dieser Fälle bei welchen das Gehör für den Dauerton gestort war dargestellt

malem Gehör für den Dauerton einer gewissen Frequenz ist auch das Gehör für kurze Töne dieser Frequenz in den Grenzen des Normalen enthalten (Abb. 3 *b*)

In Fällen der Schalleitungsstörung vom Dämpfungstyp sowie in Fällen der Hörempfindungsstörung vom ganglionären oder Hörnerventyp verliefen die Kurven der Abhängigkeit der Schwellenintensität von der Zeitdauer des Tones parallel zu den normalen Kurven (Abb 2 *a* und 4 *a*) Die Unterschiede zwischen den Schwellenintensitäten der länger und kürzer dauernden Töne sind in diesen Krankengruppen den normalen Werten gleich.

Bei den Personen, welche eine Perzeptionsstörung vom Typ der Beschädigung des Corti-Apparates aufwiesen, verliefen die Kurven der Schwellenintensitäten in den Frequenzen in welchen eine Schwerhörigkeit für Dauertöne vorhanden war mehr horizontal als die normalen Kurven (Abb. 3 *a*) Auch die Unterschiede zwischen den Schwellenintensitäten für kürzere und längere Töne waren in diesen Fällen kleiner als bei gesunden Personen Dieses entspricht dem Lautheitsausgleichphänomen, welches auch in diesen Fällen vorkommt. Wenn man nämlich die Hörschwelle eines Tones von kürzerer Zeitdauer prüft, verwendet man grössere Intensitäten, welche das kranke Ohr verhältnismässig besser empfängt.

Bei Kranken, welche die Leitungsschwerhörigkeit von Typ der elastischen Versteifung aufwiesen verliefen die Kurven für 250 und 4000 Hz Töne parallel zu den normalen Nur die Kurve des Tones von 1000 Hz hatte einen mehr horizontalen Verlauf (Abb. 2 *b*) Der Unterschied zwischen den Hörschwellen für längere und kürzere Töne war deshalb auch in der Frequenz von 250 Hz und 4000 Hz normal in der Frequenz von 1000 Hz war er dagegen viel kleiner als der normale Wert. Man muss annehmen dass in diesen Fällen das Ansteigen der elastischen Kräfte im Schalleitungsapparat die Leitung des 1000 Hz Tones von längerer Zeitdauer erschwert.

Die graphischen Darstellungen für kurzdauernde Töne, welche in der Gruppe der gemischten Schwerhörigkeit angefertigt wurden, enthalten charakteristische Züge sowohl für die Schalleitungsstörungen als auch für die Schallempfindungsstörungen.

SCHLUSSFOLGERUNGEN

1 Es besteht eine enge Abhängigkeit zwischen der Schwellenintensität des Tonreizes und seiner Zeitdauer

2. Der Hörverlust für einen Dauerton wird immer von einem Hörverlust für Kurztöne begleitet.

3. Der Verlauf der Kurven der Hörschwellen für kurze Töne ist abhängig von den gegebenen Typen der Schwerhörigkeit. Aus diesem Grunde kann man bei der Schalleitungsschwerhörigkeit den Dämpfungstyp von dem Typ der elastischen Versteifung und bei den Schallempfindungsstörungen den Cochleartyp von der Ganglion- oder Hörnervenstörung unterscheiden

Beschadigung des Ganglions oder des N Cochlearis fest Die Mittelwerte der Perzeptionsschwellen dieser Gruppe wurden in der Abb 4 *a* dargestellt Bei der Frequenz von 250 Hz ergab sich ein Unterschied der Schwellenintensi taten der Töne von 1 sek und 0 004 sek Dauer von 30 db, und der Unter schied der Schwellenintensitäten von 1 sek *und* 0 001 *sek Dauer betrug bei* 1000 Hz 52 db und bei 4000 Hz 40 db

In 13 Fällen haben wir die Hörstorung vom gemischten Typ diagnostiziert In Abb 4 *b* wurden die Mittelwerte der Schwellenintensitäten für kurzdauernde Töne von diesen Fällen dargestellt Die Differenz zwischen den Perzeptionsschwellen für die Töne von 1 sek und 0 004 sek Dauer betrug bei 250 Hz 30 db, fur die Tone der Zeitdauer von 1 sek und 0 001 sek betrug diese Differenz bei 1000 Hz 38 db und bei 4000 Hz auch 38 db

DISKUSSION

Die durchgeführten Untersuchungen bestätigten die Existenz einer engen Abhangigkeit zwischen der Schwellenintensität und der Zeitdauer der Tonreize, welche kürzer als 0 5 sek dauern Diese Abhängigkeit wurde in der logarithmischen Skala graphisch dargestellt. Bei normalhorenden Personen nähern sich die auf diese Weise erhaltenen graphischen Darstellungen für die Tone von 100–4000 Hz der geraden Linie für den Ton von 10 000 Hz jedoch nimmt die graphische Darstellung die Gestalt eines Bogens an

Die grössten Unterschiede in der Schwellenintensität zwischen einem Ton von langerer und kürzerer Zeitdauer treten in der Frequenz von 1000 Hz auf Im Bereich der tieferen und höheren Tone sind diese Unterschiede kleiner (Abb 1)

Wir berechneten die Leistung und den Druck für kurzdauernde Töne Die Kraftleistung des Schwellentones mit der Frequenz von 1000 Hz und *mit der Zeitdauer gleich* 0 001 sek *ist* 400 000*mal grösser* und der Druck 630mal grosser als die Leistung und der Druck des Tones, welcher 1 sek dauert

Das Produkt der Leistung des Tones und seiner Zeitdauer entspricht der vollkommenen Energie des Tones *Wenn wir die vollkommene Energie des* 1000 Hz Schwellentones welcher 1 sek dauert, als 1 annehmen so betragt sie für den Ton welcher 0 01 sek dauert 25 und für die Zeitdauer von 0 001 sek 400 Dagegen ist das Produkt der Zeitdauer und des Druckes des Schwellentones mit der Frequenz von *1000 Hz* eine konstante Grösse

Bei der Abkürzung der Zeitdauer der Töne mit der Frequenz 100 250 4000 und 10 000 Hz wächst die Schwellenintensitat langsamer als für den Ton mit 1000 Hz und das Produkt der Zeitdauer und des Druckes wird kleiner bei der Abkürzung der Zeitdauer dieser Töne

In allen Gruppen der Schwerhörigen wurde der Horverlust für einen Dauerton immer von einem Hörverlust für Kurztöne begleitet Bei nor

malem Gehör für den Dauerton einer gewissen Frequenz ist auch das Gehör für kurze Töne dieser Frequenz in den Grenzen des Normalen enthalten (Abb. 3 *b*).

In Fällen der Schalleitungsstörung vom Dämpfungstyp sowie in Fällen der Hörempfindungsstörung vom ganglionären oder Hörnerventyp verliefen die Kurven der Abhängigkeit der Schwellenintensität von der Zeitdauer des Tones parallel zu den normalen Kurven (Abb. 2 *a* und 4 *a*). Die Unterschiede zwischen den Schwellenintensitäten der länger und kürzer dauernden Töne sind in diesen Krankengruppen den normalen Werten gleich.

Bei den Personen, welche eine Perzeptionsstörung vom Typ der Beschädigung des Corti-Apparates aufwiesen, verliefen die Kurven der Schwellen intensitäten in den Frequenzen, in welchen eine Schwerhörigkeit für Dauer töne vorhanden war, mehr horizontal als die normalen Kurven (Abb. 3 *a*). Auch die Unterschiede zwischen den Schwellenintensitäten für kürzere und längere Töne waren in diesen Fällen kleiner als bei gesunden Personen. Dieses entspricht dem Lautheitsausgleichphänomen, welches auch in diesen Fällen vorkommt. Wenn man nämlich die Hörschwelle eines Tones von kürzerer Zeitdauer prüft, verwendet man grössere Intensitäten, welche das kranke Ohr verhältnismässig besser empfängt.

Bei Kranken, welche die Leitungsschwerhörigkeit von Typ der elastischen Versteifung aufwiesen, verliefen die Kurven für 250 und 4000 Hz Töne parallel zu den normalen. Nur die Kurve des Tones von 1000 Hz hatte einen mehr horizontalen Verlauf (Abb. 2 *b*). Der Unterschied zwischen den Hörschwellen für längere und kürzere Töne war deshalb auch in der Frequenz von 250 Hz und 4000 Hz normal, in der Frequenz von 1000 Hz war er dagegen viel kleiner als der normale Wert. Man muss annehmen, dass in diesen Fällen das Ansteigen der elastischen Kräfte im Schalleitungsapparat die Leitung des 1000 Hz Tones von längerer Zeitdauer erschwert.

Die graphischen Darstellungen für kurzdauernde Töne, welche in der Gruppe der gemischten Schwerhörigkeit angefertigt wurden, enthalten charakteristische Züge sowohl für die Schalleitungsstörungen als auch für die Schallempfindungsstörungen.

SCHLUSSFOLGERUNGEN

1. Es besteht eine enge Abhängigkeit zwischen der Schwellenintensität des Tonreizes und seiner Zeitdauer.

2. Der Hörverlust für einen Dauerton wird immer von einem Hörverlust für Kurztöne begleitet.

3. Der Verlauf der Kurven der Hörschwellen für kurze Töne ist abhängig von den gegebenen Typen der Schwerhörigkeit. Aus diesem Grunde kann man bei der Schalleitungsschwerhörigkeit den Dämpfungstyp von dem Typ der elastischen Versteifung und bei den Schallempfindungsstörungen den Cochleartyp von der Ganglion- oder Hörnervenstörung unterscheiden.

Beschädigung des Ganglions oder des N Cochlearis fest. Die Mittelwerte der Perzeptionsschwellen dieser Gruppe wurden in der Abb 4 *a* dargestellt Bei der *Frequenz* von *200 Hz* ergab sich ein Unterschied der Schwellenintensitäten der Töne von 1 sek und 0 004 sek Dauer von 39 db, und der Unterschied der Schwellenintensitäten von 1 sek und 0 001 sek Dauer betrug bei 1000 Hz 52 db und bei 4000 Hz 40 db

In 13 Fällen haben wir die Hörstörung vom gemischten Typ diagnostiziert In Abb 4 *b* wurden die Mittelwerte der Schwellenintensitaten für kurzdauernde Tone von diesen Fällen dargestellt Die Differenz zwischen den Perzeptionsschwellen für die Töne von 1 sek und 0 004 sek Dauer betrug bei 250 Hz 30 db für die Tone der Zeitdauer von 1 sek und 0 001 sek betrug diese Differenz bei 1000 Hz 38 db und bei 4000 Hz auch 38 db

DISKUSSION

Die durchgeführten Untersuchungen bestätigten die Existenz einer engen Abhangigkeit zwischen der Schwellenintensität und der Zeitdauer der Tonreize welche kürzer als 0 5 sek dauern Diese Abhängigkeit wurde in der logarithmischen Skala graphisch dargestellt Bei normalhörenden Personen nähern sich die auf diese Weise erhaltenen graphischen Darstellungen für die Töne von 100–4000 Hz der geraden Linie für den Ton von 10 000 Hz jedoch nimmt die graphische Darstellung die Gestalt eines Bogens an

Die grössten Unterschiede in der Schwellenintensität zwischen einem Ton von längerer und kürzerer Zeitdauer treten in der Frequenz von 1000 Hz auf Im Bereich der tieferen und höheren Tone sind diese Unterschiede kleiner (Abb 1)

Wir berechneten die Leistung und den Druck für kurzdauernde Töne Die Kraftleistung des Schwellentones mit der Frequenz von 1000 Hz und mit der Zeitdauer gleich 0 001 sek ist 400 000mal grösser und der Druck 030mal grosser als die Leistung und der Druck des Tones, welcher 1 sek dauert

Das Produkt der Leistung des Tones und seiner Zeitdauer entspricht der vollkommenen Energie des Tones Wenn wir die vollkommene Energie des 1000 Hz Schwellentones, welcher 1 sek dauert als 1 annehmen, so beträgt sie für den Ton welcher 0 01 sek dauert 25 und für die Zeitdauer von 0 001 sek 400 Dagegen ist das Produkt der Zeitdauer und des Druckes des Schwellentones mit der Frequenz von 1000 Hz eine konstante Grösse

Bei der Abkürzung der Zeitdauer der Töne mit der Frequenz 100 250, 4000 und 10 000 Hz wachst die Schwellenintensität langsamer als für den Ton mit 1000 Hz und das Produkt der Zeitdauer und des Druckes wird kleiner bei der Abkürzung der Zeitdauer dieser Töne.

In allen Gruppen der Schwerhörigen wurde der Hörverlust für einen Dauerton immer von einem Hörverlust für Kurztöne begleitet Bei nor

Acta oto-laryngologica 64, 11-83 (1967)

MANAGEMENT OF THE OSSICLES IN TYMPANOPLASTY

Consideration of Techniques

M. PAPARELLA
Minneapolis, Minn., U.S.A.

From the Department of Otolaryngology, University of Minnesota Hospitals, Minneapolis

In many situations of tympanoplasty it is difficult to maintain or re-establish ossicle continuity while at the same time achieve adequate exposure for removal of disease. Methods of assessing and treating the ossicles in tympanoplasties Types I, III and IV are discussed. A special situation occasionally occurs where the ossicles are normal and intact in the presence of extensive middle ear disease. A technique can be used which affords a wide middle ear exposure. It consists of removing and subsequently replacing an anterior canal skin flap, drum, malleus and incus as a "unit". In addition, special situations requiring Type V tympanoplasty using a total stapedectomy approach are discussed.

Although Zoellner and Wullstein have established useful classifications for tympanoplasty, many situations arise, depending on pathological changes, in which it is difficult for the surgeon to compensate for the condition of the ossicles and to arrive at the most appropriate physiological tympanoplasty type. Since the primary objective of tympanoplasty is always the meticulous removal of pathological tissues with the concomitant objective of preservation or reconstruction of the sound conduction mechanism, the decision can be especially difficult when there is extensive pathology in the middle ear associated with an intact ossicular chain.

In all surgical fields adequate exposure is of paramount importance to adequate management. Applying this principle to tympanoplasty is sometimes difficult. Thus, when extensive mastoid surgery must accompany tympanoplasty to eradicate infection and achieve control, it has been found preferable to use a wide postauricular exposure as advocated by Schuknecht, and not to preserve the posterior bony canal wall, but rather to expose the facial recess (posterior tympanic sinus) and sinus tympani by lowering the posterior buttress to the level of the vertical portion of the facial nerve.

Depending on the surgical approach, anatomical factors and the degree of damage to the tympanic membrane and ossicular chain, adequate visualization and removal of pathological tissues (cholesteatoma and granulations usually) in the middle ear may be difficult. Although atticotomy is helpful in exposing the epitympanum and anterior epitympanic cells, cholesteatoma may enshroud the ossicles and may be difficult to remove, especially portions located medial to the ossicles.

SUMMARY

On the basis of audiometric examinations with tones of short duration it was concluded that in healthy people the perception threshold for a tone is rising when its duration is shortening (starting from 0,5 sec duration) The difference between threshold intensity for a tone lasting 1 sec and 0 001 sec amounts to about 50 dB For 1000 Hz frequency the product of threshold pression of the tone multiplied by its duration is constant The hearing impairment for a continuous tone is always accompanied by a hearing impairment for short lived tones. With conductive hearing loss of the stifled type in cases with ganglion or acoustic nerve lesion the diminution of hearing susceptability was independent of the duration of the tone In hearing impairment of the elastically stiffened type in the frequency 1000 Hz hearing loss for tones of shorter duration is smaller In the frequencies °о0 and 4000 Hz the hearing loss was equal for longer and shorter tones. In cases of perceptive hearing loss of the type with lesion of the hearing cells, a relative improvement of hearing appeared for tones of shorter duration

LITERATUR

CASPERS, H LEHNHE, F und PLATH P 1960: Uber die Abhängigk it des Hörschw l l nsch lldru k v n der Tondau r bei Verwendung v n Kurztö en verschied ner Frequ nz. *Z Laryng* 39 7

GOLDBURT S 1964: *Nejrodinamika sluhowoj istemy cel wek* Izd Leni gr U iwers Leningrad

HARRIS, D 1937: l eak vs tot l energy in thresh lds fo rv hort to es *Acta Otolaryng* (Stockh) 4 134

KIETZ, H 1961: Der Höreindruck bei kurze Tön n. *Z Laryng* 40 112

LAN EXBECK B 1030: Messung der Hörermüdu g und Adaptation am Patiente *Z Laryng* 38 202

— 1963 *Leh bu h d r praktischen Audi m tri* G Thi me Stuttg rt

L ISCHER, E. u d ZWIS OCKI, J 1947 Th dec y of sensati n and th remainde f adaptation fter hort pure-ton impulses on the a *Acta Otolaryng* (Stockh) 35 428.

M K NOW P u d GOLD URT S 1962 A diogrammy i th wi most t diltelnosti t a lsm riaju heheg porog *Biull Ekspe Bi l* 54 21

M KOLCZI FODOR, F 1953 *Monaural l udness balance-test* d d t rmi ati n of recruitment-degree with hort und impulses. *A ta Ot laryng* (Stockh) 43 573.

NIESE, H 1965 Die Lautstärkeempfi dung Me ng und Schadli hk it n Imp lsgerä schen *Z Laryng* 44 209

P LVA, T 1036 Absolute thresholds f c ti u u d i t rrupt d pure t es. *A t Ot laryng* (Stockh) 46 129

D med M Taniewski
B gsplańskieg 39
S c in, Pol n

Eingegang n am 29 D ember 1966

of the drum or graft over the incus This limited exposure is adequate, along with palpation, to assess ossicular continuity especially in the predetermined absence of other middle ear disease

Middle Ear Pathology with Intact Ossicular Chain

The performance of tympanoplasty with mastoidectomy for chronic suppurative otitis media and mastoiditis will often reveal ossicular damage necessitating either Type III or Type IV tympanoplasty However in an occasional case the ossicles may be normal in the presence of extensive pathology in the middle ear The location of cholesteatoma or granulations may vary from primary involvement of the eustachian tube anterior areas f the middle ear regions medial to the ossicles including the oval window niche, etc The presence of an essentially normal and intact tympanic membrane with perhaps only a small marginal posterior defect or pars flaccida defect, may further complicate exposure of these areas unless normal drum os icles are purposefully sacrificed. It would be desirable if a method w re available t provide for wide middle ear exposure in such cases, and at the same time for preservation of os icular continuity

A R moval and replacement of incus and malleus

I certain instances where the ossicles are found to be normal yet there need for wid middle ear exposure a technique for removing and eplacing th incus, malleus, anterior drum remnant and anterior canal skin flap as a unit can be used The application of this technique will be dependent upon the presence of an adequate anterior drumhead remnant, preferably ne half which i necessary to upport the tympanoplasty graft t be placed subsequently After treating the middle ear this "unit is replaced and maintains its prope position chiefly through adherence of the anterior canal skin flap against the anterior bony canal wall.

After exenteration of the mastoid air cell system, the first step is to explore the incudostapedial j int to in ure that this tructure is normal A wide atticotomy i then done with a drill including removal of all of the bon wall t the lev l of the tegmen, and this area is widely saucerized. Car mu t be taken not to bump the ossicular heads causing surgical acu tic trauma r ubluxati n. The ligamentous attachments to the incus and malleu in the attic are severed. Using a mastoid curett, special care must be t ken to remove the anterior spinous proces which would otherwise impinge upon the malleus, making subsequent removal difficult. The anterior remnant of tympanic membrane is then denuded so that free bleeding occurs from the membrana propria An anterior tympanomeatal flap is raised which i similar t the posterior ... used f r approaching the stapes. A tr nsverse incision is made at ... f the cartilaginous and bony anterior canal w ... vertical incision

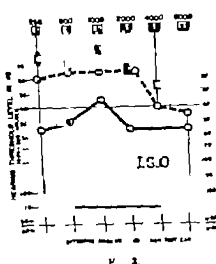
The extent or growth of cholesteatoma may vary in the middle ear Cholesteatoma may extend into anterior areas of the mesotympanum sometimes occupying a relatively inaccessible space beneath the anterior superior bony annulus and above the semicanal or into the eustachian tube Sometimes the extension of the cholesteatoma will be limited to the region of the incus, the oval or round windows or areas of the sinus tympani

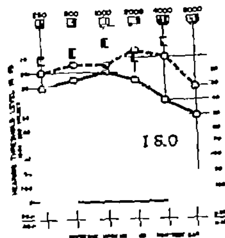
In tympanoplasty it is preferable to preserve and utilize ossicular structures whenever possible rather than to resort to the use of plastic or metallic prosthetic devices. Following the principles of adequate exposure for the treatment of middle ear pathology and the preservation of ossicles for reconstruction the intent here is to describe certain surgical techniques to help accomplish these objectives in special tympanoplasty situations. In addition suggestions of technique in managing the ossicles in more typical situations of tympanoplasty will be made Representative case reports and audiograms have been selected for illustration The variability of middle ear pathological changes seems to warrant individualization of approach for the eradication of infection and the maintainance or restoration of function

Myringoplasty—Tympanoplasty Type I

In many cases of myringoplasty there is sufficient preliminary clinical information such as audiometric studies with patch tests, to provide for clear cut indications of tympanoplasty type Relative to the causation of the perforation many patients may also have defective ossicular chains necessitating exploration A common method of exploration is to elevate a tympanomeatal flap as in stapedectomy and to reflect the drum with its fibrous annulus forward The removal of sufficient bony annulus lateral to the oval window provides good visualization of the stapes and the long process of the incus This method is especially useful if other areas of the middle ear require exploration The denudation of outer drum head epithelium prior to elevation of the tympanomeatal flap helps to facilitate this surgical step in preparing a recipient site for subsequent grafting

If such an exploration is done it is possible to inadvertently convert a myringoplasty to a Type II tympanoplasty or myringoincudopexy This is especially true if excessive manipulations of the long process of the incus are necessary to assess ossicular damage In such a case the long process and perhaps part of the body of the incus, depending on the extent of bony removal will be uncovered by bone and the drum or graft may attach itself to the incus To circumvent this possibility and the consequent slight loss of auditory acuity it is best when exploring the ossicles in myringoplasty to leave the drumhead and fibrous annulus in their normal positions. By using a fine drill the bony annulus in the region of the long process and oval window area can be removed, permitting an exposure to this area which upon replacement of the canal skin flap does not result in a draping





F 3.   Fig. 6.

Fig. 3. Gra lation tissue nd stratified squamous epithelium practically filled the middle ea of this patient wh had bro ic otitis media nd mast iditis. The pathology xt nded parti lnt the eustachia t bal orifice but th ossicles were intact and nor mal.—Remov l nd replacement of unit (incus, malleus, drum nd anterior canal flap) S. B. Ag 20 ♀ O—O, pre-operati air conduction thresholds O---O 24 mos. post-operat- th conduction thresholds [pre nd post-operath bone conduction thresholds. 94% d scrimination—pre- nd post-operati

Fig. 6. A small holesteatomatous cyst wa present I the incus regiod in thi patient Anterior mesot mpa um ppeared normal nd co trol window in the trans re- ealed normal mucosal li i g The incu removed t achles limited exposure and as replaced I H ormal position. — Removal nd repl cement of incus. H. B Ag 41 ♂ O—O pre-operath ai conduction threshold ; O---O 18 mos. post-operatis air con- duction thresholds; pre- nd post-operath bone conduction thresholds. 91% dis- crim nation—pre nd post-operath

disease the "unit is replaced in its normal position maintained by the adherence of the anterior can l skin flap against the bony canal The lenticular process of the incus is placed upon the stapes head and this area i carefully packed and secured with gelatin foam. The graft (skin or f scia) is carefully placed upon the anterior drumhead remnant (recipi ent site) and covers the middle ear Appropriate packing and wound closure completes the procedure

Although thi procedure may be time consuming (requiring perhaps an additional hour of operative time) it provides wide exposure while at the same time th middle ear space and ossicular continuity are preserved (Fig 3)

B. *R moval and replacement of malleus*

If the pathology is localized only in the eustachian tube or anterior areas of the middle ear a modification of the above described technique can be

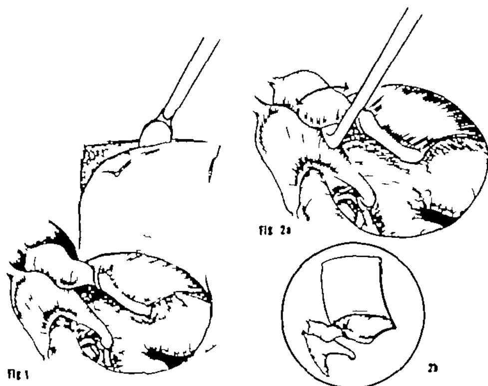


FIG 1 This drawing shows the incision for the anterior tympanomeatal flap prior to removal of the malleus, incus, anterior drum remnant and anterior canal skin as a "unit"

FIG. 2 *a* The tensor tympani tendon is severed close to the malleus. 2 *b* The "unit" is now free and consists of the anterior canal skin, the anterior drum remnant the malleus and incus in continuity which is placed in Ringer' solution to be replaced in the middle ear subsequently

completes the rectangular *flap which is reflected downward (Fig 1)* Special care must be taken to elevate the anterior fibrous annulus from its sulcus by passing a fine elevator or other appropriate instrument beneath it so as not to perforate the drumhead in this location The final step is to free the "unit" by cutting the tensor tympani tendon This is done by passing a right angle knife between the malleus and incus, staying close to the under surface of the malleus (Fig 2 *a*) Sometimes appropriate positioning of the patient s head will allow direct visualization of this structure which can be severed more easily The "unit" consisting of the incus, malleus, denuded drumhead remnant and anterior canal skin flap is then placed in heparinized blood or Ringer s solution until later in the procedure (Fig 2 *b*) The "unit" may be held in the finger tips and bits of granulations may be cleaned off if *necessary Occasionally subluxation of the incudomalleolar* joint may occur in removal which does not represent a serious problem

With the "unit" thus removed the entire middle ear can be well seen and treated If there is a prominent anterior bony canal wall it can be drilled down with a bone cutting burr After the eradication of middle ear

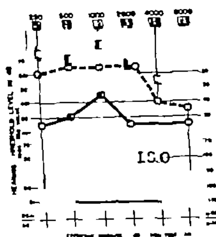


Fig. 5.

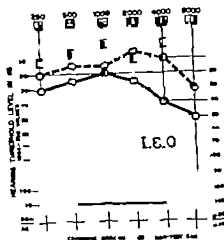


Fig. 6.

Fig. 5. Granulation tissue and stratified squamous epithelium practically filled the middle ear of this patient who had chronic otitis media and mastoiditis. The pathology extended partly into the eustachian tubal orifice, but the ossicles were intact and normal.—Removal and replacement of unit (incus, malleus, drum and anterior canal flap) S. B. Ag. 30 ♀ O—O pre-operative air conduction threshold O--O 24 mos. post-operative air conduction threshold pre- and post-operative bone conduction thresholds. 96% discrimination—pre- and post-operative.

Fig. 6. A small cholesteatomatous cyst was present in the incus region in this patient. Anterior mesotympanum appeared normal and round window in the drum revealed normal mucosal lining. The incus was removed to achieve limited exposure and replaced in its normal position. — Removal and replacement of incus. H. B. Ag. 41 ♂ —○ pre-operative air conduction thresholds O--O 18 mos. post-operative air conduction thresholds pre- and post-operative bone conduction thresholds. 94% discrimination—pre- and post-operative.

disease the unit is replaced in its normal position, maintained by the adherence of the anterior canal skin flap against the bony canal. The lenticular process of the incus is placed upon the stapes head and this area is carefully packed and secured with gelatin foam. The graft (skin or fascia) is carefully placed upon the anterior drumhead remnant (recipient site) and covers the middle ear. Appropriate packing and wound closure completes the procedure.

Although this procedure may be time consuming (requiring perhaps an additional hour of operative time) it provides wide exposure while at the same time the middle ear space and ossicular continuity are preserved (Fig. 3).

B. Removal and replacement of malleus

If the pathology is localized only in the eustachian tube or anterior areas of the middle ear a modification of the above described technique can be

used in which the incus is left in place The removal of the malleus, drum head remnant and anterior canal skin flap can also be done through an endural incision especially if mastoid surgery is not necessary in cases where the disease is limited to the middle ear cleft The atticotomy is widely done and the ligamentous attachments to the malleus alone are severed to include the tensor tympani tendon The incudomalleolar joint is disarticulated leaving the incus undisturbed and the malleus, anterior drumhead remnant and anterior canal flap are removed as a unit As described above it is easier to de epithelize the drumhead prior to its removal This technique permits a wide exposure to the eustachian tubal area and anterior epitympanum After removal of disease in the middle ear the head of the malleus is reapproximated to the body of the incus and its position is maintained by the anterior canal skin flap which holds it securely in place The drum head remnant then helps to provide support and vascularization to the graft which is then placed (Fig 4)

C. *Removal and replacement of incus*

Schuknecht has demonstrated the possibility of removing the incus and replacing it in its normal position in the surgical treatment of Bell s palsy without subjective hearing loss resulting perhaps amounting to only five or ten decibels. Subsequently I have used this technique in cases of facial nerve decompression for Bell s palsy in which simple mastoidectomy and a decompression of the vertical portion were done first Since a lesion was not seen in the vertical course of the nerve the posterior incudal ligament was severed and the incudostapedial joint disarticulated, whereupon the incus was easily delivered through the antrum posteriorly and placed in Ringer s solution for later replacement This afforded an excellent view of the entire horizontal portion of the facial nerve The nerve was then decompressed up to and including the geniculate ganglion. Afterwards the incus was replaced in its normal position and gelatin foam was packed around the reapposition site of the lenticular process and head of stapes The incus is secured in its normal position by the presence of a normal fossa incudis serving as a receptor for the short process and sufficient bony annulus to help provide lateral support

This same technique can be applied in certain unusual cases of tympanoplasty where a small cholesteatoma for example may be localized to the region of the incus and cannot be viewed or removed in the presence of the incus In such a case the incus can be removed and replaced subsequently with little discernible hearing loss resulting providing the cholesteatoma has not destroyed the fossa incudis and a bony bridge can be left (Figs. 5 and 6)

Missing Long Process of Incus

The most common congenital and acquired lesion of the incus is a loss of part or all of its long process. In the performance of tympanoplasty it

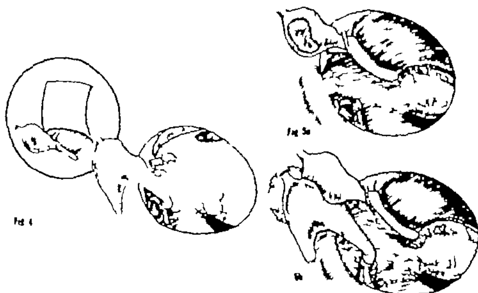


Fig. 4. Thi modificatio of the previ us procedure i used when disease i localized in the nterior epitympa ic or mesotympanic rea t include the eustachian tube. In this case the terior canal skin, terior drumhead remna t and malleu ca be removed nd replaced later. The incus i left in its ormal positi n.

Fig. 5 a. The incus has been removed. 5 b. After the pathology medial t the incu ha been removed, the incu i replaced i its normal position and i supported by gelfoam.

I common to find either the lenticular process or more of the long process of the incus missing due to erosion. After treating the middle ear it is desirable to use the incus, if possible to re-establish ossicular continuity. If only the lenticular process of the incus is missing (Fig. 7 *a*) it is useful to excise a small rectangular piece of cartilage from the pinna or a small piece of bone from the mastoid area. A small cup like depression is made on one sid with a fine stapes bur which will serve to receive the head of the stapes. This piece of cartilage (Plester) or bone is then interposed between the long process f the incus and the head of the stapes. A slight over correction is desirable so that the long process is pushed slightly outward from the stapes head (Figs. 7 *b* and 7 *c*)

If on the other hand, a larger portion or all of the long process of the incus is missing (Fig 7 *a*) it is preferable to perform incus transposition a described by Guilford (1965) In this case the tenso tympani tendon i transected, to allow the manubrium of the malleus to lift up from the promontory. A small cup like depression i then made on the under surface of the body of the incus using a small bone cutting bur. The short process of the incus i positioned beneath the manubrium and the head of the st pes makes contact with the depression on the body of the incus (Fig 7 *d*). Gelatin f am i then packed around the area for stabilization and the incus

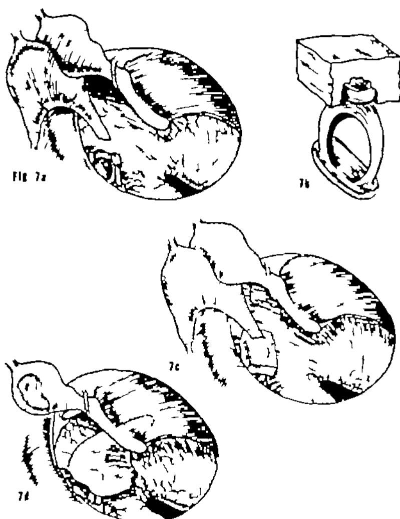


FIG. 7 a Tl l d awl g h w *missing l ticula process The dotted line indicates* tl pos ibility f ro l n f tl lo g proce up t th bod f the l cu 7 *b* Wh *nly* tl lent ular process f the l cu l mi sing, the f ll wing techniqu l preferred. A mall rect gul piece of cortic l bo l wedged between the he d f the stapes and tl long proc f th l cu A small d pres l n l mad n on sld with a bone utti g burr whl h rec l es the head f the t pe A light c correction de irabl *d* Wh n m re of th l g proces of th l s missl g tra spo itl n f the Incu procedure l d The hort proce f the incus l positi ed beneath the m l lum f th malleu

is thus balanced on the head of the stapes and undergoes ballottement easily (Figs 7 *a* 7 *d* and 8)

Type III and Type IV Tympanoplasty

When a patient presents with chronic mastoiditis and chronic suppurative otitis media often either Type III or Type IV tympanoplasty is necessary In the presence of extensive malleus and incus damage it is still preferable in many cases to perform Type III or Type IV tympanoplasty rather than to attempt tympanoplasty conversion This is especially true when complete

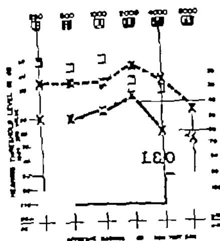


Fig. 8.

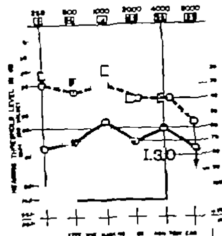


Fig. 10.

Fig. 8. The middle ea w normal in this patient, however the long process of the incu had been eroded due t previou disease Incus transposition wa performed — Tran position f Incus. R. W Ag 12 ♀ — pre-operative l conduction thresholds; 12 mos. post-operative lr conduction thresholds] pre- d post-operativ bone conduction thresholds. 90 discrimination—pre- nd post-operativ

Fig. 10. This patient had history of hronic titis media nd chronic mast iditis A Type IV tympanopl sty ith mastoidect my w don t the first peration. Tympanosclerosis invaded the footpl t of the stapes nd w mobilized with improved heari g resulting fter surgery which, however regressed t a even greater conduc t loss due t refixat on. Eight months later the previousl placed graft w elev ted nd the footplat was caref ll removed nd the graft was then f agmated int the open ov l window cont l ing mall mount f g latin foam Thi then represents Type V tympanoplasty using total st pedectomy pproach. — Type V (stapedectomy) tympanopl sty V F Age 45 ♀ O—O pre-operativ l conductio thresholds: O—O, 10 mos. post-operati l conduction thresholds; [post-operati bone conduction thresholds (sl ghtl better tha pre-operati) 100% discriminat on post-operati 94 pre-operati e.

mast idectomy accompanies tympanoplasty Until it becomes mechanically nd practically more feasible to convert tympanoplasties Type III and IV int lesser tympanoplasty types (f r example physiological Type II tympanopla ty) it appears pref rable at this time to continue to apply the proven Type III and Type IV methods. It is possible that with the passage of time superior results may consistently be proven with conversion tympanoplasty however at the present time such techniques are not as well established

Con ideration and treatment f the eustachian tube the hypotympanic lining and the oval and round windows are of importance in all cases, and a uccessful result will depend to a large extent upon the proper management of these areas.

In eith r Type III or Type IV tympanoplasty critical area to be con-sid red are the facial recess (post rior tympanic sinu) and sinus tym-

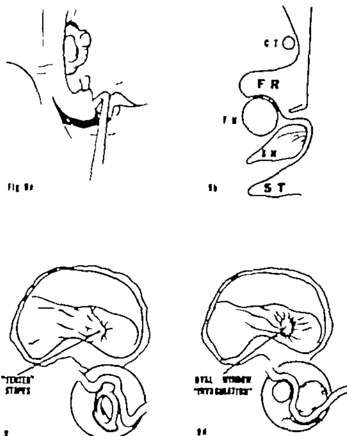


Fig 9 a Th si us tympani I indicated by the dotted lin The instrument i palpating th sinus tympani which xist medial to the pyramidal process. 9 b This diagrammatic cro secti al view was drawn from a t mporal bon ection to indicate rel ti nship of th faci l recess d inus tympani C.T Chorda T mpa i F.R Facial Recess F N F ci l N rve S.M Stapedi l M scl within th pyramidal proces S T Sinu Tymp ni 9 Th "t nted st pes as seen in Type III tympanopla ty 9 d. The graft i in gl ated with a tiny pledget f cotton in T pe IV tympan plasty in which the footpl te i present nd in Type V tympan pl ty in which the footplat is not present

pani These areas should be explored and exposed fully in order to remove pathology which is often hidden and also to provide the effect of a *wide saucerization around the oval window so that graft adherence to* the oval window will take place and the optimal effect of sound pressure dif ferential between the windows will be promoted It is equally important to treat these areas whether the tympanoplasty is being performed postauri cularly in combination with mastoidectomy or endaurally in which case a wide attico antrotomy is often necessary (Figs. 9 *a* and 9 *b*)

In either Type III or Type IV tympanoplasty it is always important to meticulously remove any pathological tissues which exist in the region of the oval window Schuknecht has demonstrated the importance of tenting the stapes in Type III tympanoplasty (Schuknecht) This tent is produced by the fact that the head of the stapes is slightly higher than the

level of the horizontal portion of the facial nerve. It is important to insure that the Fallopian canal is completely de-epithelialized along with the medial attic wall since graft adherence is necessary in these areas.

In performing Type IV tympanoplasty the graft must be firmly invaginated against the footplate (Schuknecht) This is accomplished by using a tiny cotton pledget which carefully pushes the graft into the oval window niche. It is extremely important that all mucosal remnants are removed from the oval window niche and that an adequate recipient site exists on the inferior margin of the oval window in Type IV cases. Again, the Fallopian canal must be entirely denuded to receive the graft (Figs. 9 *c* and 9 *d*) There are of course many other factors which have an important bearing on the ultimate functional result in tympanoplasty which are too numerous to discuss in this brief report

Based on personal experience in more than 230 cases of Type III or Type IV tympanoplasty usually in combination with mastoidectomy and obliteration, we have found that at least 50% of all cases will achieve a serviceable hearing level of forty decibels (ISO) or better

Type V Tympanoplasty

Occasionally tympanosclerosis will be found in the oval window causing partial or complete fixation of the stapes footplate. In such cases it is preferable to thoroughly eradicate all infection in the mastoid air cell system and middle ear and to perform Type III or Type IV tympanoplasty as indicated Although tympanosclerosis should be safely removed from the oval window when possible the footplate must be left intact and the inner ear must not be exposed at the time of initial surgery In some instances it will be possible to mobilize the stapes and to achieve an adequate functional result Recurrent stapes footplate fixation will commonly occur and the improved hearing will be short lasting Complete stapedectomy may be considered after many months of observation to insure that the middle ear and mastoid are completely dry without evidence of recurrent or residual infection. In three cases, after exploration revealed footplate fixation, Type IV tympanoplasty was converted into Type V tympanoplasty via a total stapedectomy approach.

The previously placed graft is elevated and the footplate is carefully removed as in stapedectomy for otosclerosis. A small pledget of gelatin foam moistened in Ringer's solution is placed in the oval window first. The graft is then carefully repositioned and invaginated with a cotton pledget as in Type IV tympanoplasty (*Fig. 9 d*) The cotton pledget is left in place for two weeks along with the packing.

Although these three cases have been successful (Fig. 10) at least 18 months after surgery at the time of the present writing, they are mentioned here primarily to illustrate the technique and not long term results. It is hoped that further comparative reports evaluating Type V tympano-

plasty using both the stapedectomy (Type V b) approach and the fenestration of the horizontal semicircular canal (Type V a) approach will appear in the literature

SUMMARY AND CONCLUSIONS

In myringoplasty or Type I tympanoplasty it is often necessary to evaluate the ossicular chain during surgery. When the situation permits it is preferable to leave the drum and posterior annulus fibrosis in their normal positions and to explore the incudostapedial joint and oval window area through an appropriately positioned window in the bony annulus

There is no question that further advances in tympanoplasty will result in improvement of techniques which can be adapted to a wider group of patients. At the present time and in the absence of proven and consistent results with middle ear prosthetic devices, it seems preferable to utilize remnants of existing ossicular structures whenever possible

In certain cases extensive pathology can co-exist with normal ossicles in the middle ear and it becomes important to achieve a wide exposure with a secondary objective of re-establishing ossicular continuity. A technique consisting of removal and replacement of the incus, malleus, anterior drum head remnant and anterior canal skin flap as a unit has been described. This can be modified to include only removal and replacement of the malleus, drumhead and anterior canal flap when necessary

When pathology is localized medial to the incus region it is feasible to remove a normal and intact incus for exposure and to replace it in its normal position with very little resultant functional loss. A missing lenticular process of the incus is best managed by inserting a wedge of cartilage or bone between the long process and the head of the stapes. When more of the long process of the incus is missing it is preferable to resort to incus transposition as described by Guilford

Whenever Type III or Type IV tympanoplasty is performed it is always important to expose the facial recess and sinus tympani for the complete removal of disease in addition to providing a wider recipient site for grafting. Proper management of these areas also provides for improved phase differential characteristics between the two windows. In performing Type III tympanoplasty it is always desirable to achieve a "tenting" of the stapes as stressed by Schuknecht. In Type IV tympanoplasty it is equally important to invaginate the graft tightly into the oval window against the footplate of the stapes with a tiny pledget of cotton

Fortunately stapes fixation is rarely encountered in tympanoplasty. When fixation does occur tympanosclerosis is usually the cause. After the mastoid and middle ear infection have been thoroughly controlled surgically and after a minimum period of six to twelve months of observation have passed postoperatively to help insure that residual or recurrent infection are not present it is feasible to perform Type V tympanoplasty through a stape

dectomy approach. In such a case, the footplate is carefully removed, gelatin foam is placed into the oval window and the graft is carefully invaginated into the oval window as in Type IV cases.

Although this discussion has been mainly concerned with management of the ossicles in tympanoplasties, it is never possible to separate these technical considerations from other important associated considerations. In all cases a functional eustachian tube and an adequately lined hypotympanum are essential prerequisites for the attainment of favorable hearing results.

ZUSAMMENFASSUNG

In vielen Situationen von Tympanoplastiken ist es schwierig, die Gehörknöchelchenverbindungen zu erhalten oder wieder herzustellen und zur selben Zeit eine ausreichende Freilegung für die Entfernungen der Pathologie zu erzielen. Methoden für die Beurteilung und Behandlung der Gehörknöchelchen in Tympanoplastiken von Typ 1, 3 und 4 werden diskutiert. Eine besondere Situation besteht manchmal, wenn die Gehörknöchelchen normal und in Kontakt sind in der Gegenwart von ausgedehnter Mittelohrerkrankung. Es wird eine Technik beschrieben, welche eine weite Mittelohrfreilegung erzielt und aus der Entfernung und anschliessenden Wiederverwendung des vorderen Gehörgangslappens, Trommelfells, Malleus und Inkus als eine Einheit besteht. Zusätzlich werden besondere Situationen, die eine Tympanoplastik Type 5 erfordern, diskutiert.

REFERENCES

Schuknecht, H. F. Personal Correspondence.
Plester, D. Personal Correspondence.
Guilford, F. R. 1965. Repositioning of the incus. *Laryngoscope 75*, 236.

Dept. of Otolaryngology, University of Minnesota Hospitals, Minneapolis, Minn., U.S.A.

Received November 1, 1966

Acta oto-laryngologica 64 95–104 (1967)

HABITUATION TO REPEATED ROTATORY STIMULI (CUPULOMETRY) AND THE EFFECT OF ANTINAUSEA DRUGS AND ALCOHOL ON THE RESULTS

G Aschan

Uppsala Sweden

From the Department of Otolaryngology Akad miska Sjukhuset, Uppsala

Repeated rotat ry tests performed as cupulometry every second d y five times resulted in response decline which was significant only for th sensation cupulogram. This response decline was more pronounced the stronger th rotatory stimuli used.

In a seco d series the cupulogram before and two hours after the intake of four different antinauseadrugs were compared. A fifth test was performed with placebo tabl t The tw nty subjects tested all five po sibilities. The statistical nalyses sh wed that in th sensation cupulogram the placebo test had an h n effect that very conclusion regarding therapeutical ffect o post-rotat ry sensation was valueless. Regarding the ystagmus cupulograms the results were somewhat better but the placebo test showed that the more frequent rotatory stimuli in lved i the second series resulted in a slight response decline Only on drug consisting f methylscopolaminl nitras 0.5 mg a d pentymalum 50 mg (Veryl® Pharmacia) reduced the post-rotatory nystagmic respo se significantly

I fighter pilots with a marked respo se decline to rotatory stimuli, ttributed t their occupational training, alcohol int xication made the response declin lisappear during moderate intoxications of up to 1.0 per m lle maximal blood alcohol concentration From experiment l d t nd the results of this investigation it seems reasonable to assume that lcohol acts a slight an esthetic elimin ting the response decline which it thus seems justif d t call habituation. It must be of central orig and probably is the result f n alcohol depressor effect above the brain stem and cerebella level.

Since Bárány (1907) introduced the rotatory test into the clinical ex mination his original method has undergone several modifications. The reason for this has been that the original technique involved two accelera t ry stimuli too close t each other that these stimuli were very strong nd that a repeated test showed a decreased response regarding the dura tion f post rotatory nystagmus (Dodge 1923 Holsopple 1924 Fischer

Thi in stigation w pported by the S edish Medical Research Council, Project 22X-36-02.

1928 Mittermaier 1952) In 1949 van Egmond, Groen & Jongkees introduced a new rotatory technique called cupulometry They stated that cupulometry following a Bárány test showed a decreased response which persisted for several days It has also been shown that individuals who in their daily work are exposed to repeated rotatory stimuli for example fighter pilots (Aschan 1954) ballet dancers and figure skaters, exhibit a decrease in the duration of their post rotatory nystagmus and sensation when tested with cupulometry

In animal experiments Hamberger & Hydén (1949) and Hood & Pfaltz (1954) showed a gradual decrease in post rotatory nystagmus following moderately intense rotatory stimuli

Hallpike & Hood (1953) recommended the term "response decline" for the phenomenon of decrease of the post rotatory nystagmic response following repeated stimuli This was to avoid confusion with terms like fatigue adaptation or habituation, used in cochlear physiology As long as the nature of the phenomenon is not known it would seem advisable to follow their recommendation The term response decline" will be used in this paper to denote a decrease in both post rotatory sensation and post rotatory nystagmus

The phenomenon of response decline to rotatory stimuli has attracted a great deal of attention during recent years, for example in training astronauts, and for this reason it is of great interest to try to investigate its nature Some interesting aspects of cupulometry were discussed by de Wit (1953) who found in his investigations that sensation cupulograms for sailors who were chronically seasick differed from the value obtained from sailors who were less troubled in this respect de Wit also claimed that the prophylactic effect of antinausea drugs could be demonstrated by changes in the sensation cupulograms before and after medication

One aim of the present investigation was to determine whether the rotatory stimuli involved in repeated cupulometric examinations as such could produce a response decline Two phenomena follow a rotatory stimulus, viz a sensation of turning and post rotatory nystagmus. They can be recorded simultaneously and as some of the investigations mentioned in the introduction concern only sensation, and others only the duration of nystagmus, it should be of interest to study them both at the same time The second aim of the investigation was to study the effect of antinausea drugs on the cupulogram It was considered that such an investigation might answer the question of whether cupulogram changes after antinausea drug medication could give reliable information in the clinical testing of such drugs. The third part of the investigation deals with a special group of test subjects, viz. fighter pilots with a marked cupulometric response decline Alcohol intoxication had a marked influence here on a repeated cupulometric test and these results were included as it was considered that they may be of value for the discussion on the nature of post rotatory response decline

MATERIAL

Twenty subjects of both sexes were used in the first two parts of the investigation. Most of them were medical students, 19 to 25 years old. The subjects were selected, all having a negative otoneurological history normal caloric reactions, normal audiograms, and in other respects normal otoneurological findings. It was also checked that they all had a normal EEG The third part of the investigation concerned ten well-trained fighter pilots. They all showed in cupulometric tests the marked response decline on rotatory stimuli, which has been described by Aschan (1954) Out of a group of a hundred pilots it was found that these ten had the most marked cupulometric changes and that the changes could be attributed to their training

METHODS

The rotatory test

All rotatory tests were carried out in the rotating room described by Aschan, Pettersson & Ofverholm (1953) The rotation of this room is under electronic control and the rotatory stimuli applied could be produced and reproduced with a very high degree of accuracy Every rotatory test, demonstrated as a cupulogram, involved 6 rotations at a constant speed of 15 30 and 60 /sec, with rotation both to the right and to the left. The acceleration was always 0 45 per second per second and the deceleration 20 per second per second Every second subject always started with rotation to the right, and the others with rotation to the left this was done to avoid the difference that may f llow when the turning is always started in one direction, as pointed out by Aschan *et al.* (1952)

Nystagmography

Nystagmus was always recorded by the method described by Aschan (1955) and Aschan *et al* (1956) Only horizontal electrodes were used, and thus only nystagmus in the plane of rotation was recorded. All recordings were made with closed eyelids. A calibration for 10 eye movements in the plane of the electrodes was performed at the start and end of an experiment. The subjects were asked to state when their sensation of turning had ended, and thi was marked on the record. Thus the duration of post-rotatory sensation and nystagmus could be read directly

Plan of the Investigation

A Twenty normal ubject had one cupulogram taken every second day until they had five consecutl recordings.

B One month l ter the same ubjects had one cupulogram taken Immediately afterwards they were given ne out of five possible drugs. Two

hours later a further cupulogram was taken Two to three days later the same program was repeated with a new drug until every subject had been tested with the five following drugs

1 Amosyt* Leo Sweden (Diphenhydramini theoclas, Dimenhydrinate 0 1 g)

2 Allergisan* Pharmacia Sweden (Chlorphenamini maleas, Chlorpheniramine Maleate 4 mg)

3 Veryl* Pharmacia Sweden (Methylscopolamini nitras, Hyoscine Methonitrate Methscopolamine Nitrate 0.5 mg + Pentymalum, Amobarbitalum Amylobarbitone 50 mg)

4 Hysco* Pharmacia Sweden (1 Hyoscyamini Camphoras 0 8 mg + 1 Scopolamini Camphoras 0 2 mg)

5 Blind tablet

The substances tested were chosen so that numbers one and two represented antihistaminic drugs advertised as having an antinausea effect Preparations three and four were not of the antihistamine type but were also advertised as antinausea drugs. The dose chosen was such that in between the tests the subjects were able to perform their normal duties. The test conditions were arranged in such a way that every fifth subject started with the same drug Thus it was possible to have a systematic variation which was, however unknown to the test subjects.

C The ten fighter pilots first had their cupulogram taken then they were given 15 cl whisky on an empty stomach and 100–140 minutes after this intake a second cupulogram was performed In addition to this test, the blood alcohol concentration was analysed at intervals of about 40 minutes, and the positional alcohol nystagmus tested as described in a series of papers by Aschan *et al* (1955–1964)

RESULTS

A *Cupulogram repeated every second day five times* The duration in seconds of the post rotatory sensation and nystagmus was noted and the values obtained were treated together independently of the turning direction Thus $n = 40$ when calculating the mean values except for some minor variations due to technical difficulties in a few records which can be disregarded Table 1 gives the values for the sensation cupulograms. The corresponding values for the nystagmus cupulograms are given in Table 2 The statistical analyses followed the same principles as in a previous investigation (Aschan *et al* 1952) The value for one single cupulogram was subtracted from the corresponding value for the previous one The differences were then added with a plus sign for higher values and minus for lower values The mean was calculated for all these values at the five different tests *Using this method the differences between the first sensation* cupulogram and the subsequent ones are significant, the difference between

TABLE 1. *Duration of post rotatory sensation.*

Cupulometric mea[illegible] lues from fi[illegible] consecutiv[illegible] tests with 48 hours int[illegible]val [illegible] 20 normal subjects.

Cupulogram No.	Rotation speed: 60 °/sec M ± ε(M)	[σ]	30 °/sec M ± ε(M)	[σ]	15 °/sec M ± ε(M)	σ
I	20.3 ± 1.1	6.8	18.1 ± 0.9	6.0	7.9 ± 0.8	3.7
II	18.7 ± 0.9	5.9	13.2 ± 0.9	5.4	7.4 ± 0.4	2.7
III	16.1 ± 1.1	6.9	10.9 ± 0.8	5.1	5.7 ± 0.6	4.1
IV	14.9 ± 0.9	5.5	9.3 ± 0.4	2.6	4.8 ± 0.2	2.1
V	13.7 ± 0.8	5.2	8.0 ± 0.5	3.2	3.6 ± 0.4	2.8

the first and the second being -5.00 ± 1.27 and between the first and the last 0.66 ± 1.65. The response decline in the sensation cupulogram is also more pronounced with higher stimuli, i.e. a higher speed of rotation. With regard to repeated nystagmus cupulograms no definite differences were shown. The largest difference was found between test number 1 and test number 5, this being -4.20 ± 1.07.

Tables 1 and 2, complemented with the statistical treatment just described, thus show that the sensation cupulogram gradually develops a response decline which is not observed in the nystagmus cupulograms.

B) *Experiments with normal subjects before and two hours after medication.* The values obtained are given in Tables 3 and 4. From the values there seem to be a response decline both before and after the administration of the different drugs. However, the control or placebo test immediately reduces the possibility of drawing any definite conclusion. The material was treated statistically following the rules described, and the result is as follows.

TABLE 2. *Duration of post rotatory nystagmus.*

Cupulometric mea[illegible] lues from fi[illegible] consecutiv[illegible] test with 48 hours inter[illegible] the same 20 normal subject[illegible] Table 1.

Cupulogram No.	Rotation speed: 60 °/sec M ± ε(M)	[σ]	30 °/sec M ± ε(M)	σ	15 °/sec M ± ε(M)	σ
I	37.1 ± 2.1	10.5	28.7 ± 2.2	14.2	11.8 ± 1.3	8.1
II	31.4 ± 1.4	8.7	23.1 ± 1.8	11.2	11.0 ± 1.2	7.6
III	31.7 ± 1.9	11.7	23.2 ± 2.7	17.1	12.9 ± 0.9	5.7
IV	31.8 ± 2.2	14.3	22.7 ± 2.1	13.1	10.1 ± 1.1	7.0
V	31.3 ± 1.6	10.1	22.5 ± 1.6	10.3	8.3 ± 1.0	6.1

TABLE 3 *Sensation cupulogram values from 20 normal test subjects before and two hours after the administration of five different drugs no 5 being a placebo test*

Drug number	1	2	3	4	5
Rotation speed /sec					
60	17 4±1.8	17 6±1 7	18 2±1 6	17 1±1 6	23.1±2.4
30	11 7±2.0	12.0±0 9	16.5±2.0	10.5±1.3	14.3±1.2
15	5 0±2.1	6.2±0.7	6.0±1 1	5.4±0.9	6.9±2.0
Values two hours after drug administration					
60	15 2±2.3	14 7±1.3	14 0±2.5	15.1±1 1	18.0±1.2
30	9 0±0.7	8. ±2.0	9.3±1 1	9 4±1 7	11 6±1 7
15	5 1±1 7	5.6±0.9	4 1±0.6	4.5±0.5	6.2±1 6

TABLE 4 *Nystagmus cupulogram values obtained simultaneously with the sensation values presented in Table 3*

Drug number	1	2	3	4	5
Rotation speed, /sec					
60	32.6±1 0	31 9±1 7	30.9±2.1	33.5±2.3	37.6±3.1
30	24.7±2.1	21.3±1 1	23.2±1 4	23.5±1 7	28.1±2.9
15	9 4±1 7	10.2±2.0	11.2±1 7	10 1±2.0	11.5±1 0
Values two hours after drug administration					
60	27.3±2.3	24.7±2.3	25.0±1.5	30 0±1 7	32.2±1.2
30	20.3±1 0	18.0±1 7	18.3±1 9	22.0±1.9	27 6±3.1
15	7.3±0 1	9.3±1 1	9 1±1 2	10.5±0.9	11 4±0.7

Sensation cupulogram

Drug (no) 4 −1 33±0 87 1 −1 60±0 78 2 −2.25±0 84 5 −2.83± 0 81 (placebo) and 3 −4 43±0 01

Nystagmus cupulogram

Drug (no) 4 −1.53±1 36 5 −2 08±1 80 (placebo) 2 −3 80±1 14 1 −3 00±1.20 and 3 −4.27±1 12.

In the sensation cupulograms only drug no 3 and the control or placebo test gave "significant results In the nystagmus cupulograms, however the blind test and one of the drugs did not show any significant differences. Drugs nos 1–3, however showed in the nystagmus cupulogram a significant reduction of the duration of the post rotatory nystagmus two hours after their intake In the sensation cupulograms it is clear from the result of the placebo tests that the values obtained for drugs 1 2 and 4 give us no information at all from the clinical point of view Regarding the nystagmus

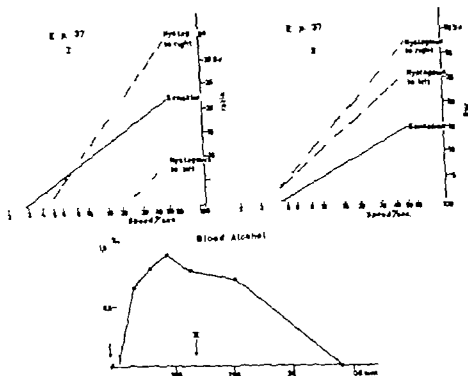


FIG. 1 Cupulogram before (I) and during alcohol intoxication (II) in a well trained fighter pilot. Before intoxication he showed marked response decline in his left-beating nystagmus (I) which disappeared during intoxication. The graph at the bottom gives his blood alcohol concentration curve following one single dose of 150 ml of whisky

cupulograms the results for drugs 2, 1 and 3 can be attributed to the drugs given and not a response decline Drugs 4 and 5, on the top and bottom of the above list respectively can only be correctly evaluated when the result of the placebo test is considered.

C. *The effect of alcohol intoxication on test subjects with a marked response decline in their cupulograms* This part of the investigation was included because of a chance observation in a fighter pilot As the initial observation could be confirmed in later experiments—with a few reservations mentioned later—and as the initial cupulogram values differed greatly from one test subject to another the results cannot be described as in series A and B. The original experiment will serve as an illustration

A 21 year-old well-trained fighter pilot showed the cupulogram marked I before alcohol intoxication (Fig. 1) The marked response decline in his left-beating nystagmus could be explained by a habit of rolling to the right (Aschan, 1951) Two hours after the intake of 15 cl whisky and with a blood alcohol concentration of 0 9‰ his response decline in the nystagmus cupulogram had disappeared (Fig 1 graph II) Four hours

after the alcohol intake and with a blood alcohol concentration of 0.51‰ his nystagmus cupulogram was the same but his sensation cupulogram gave such values that they could not be reproduced graphically. Two days later he behaved as before the intoxication.

Eight out of ten test subjects in whom the maximal blood alcohol concentration ranged from 0.52 to 0.95‰ all showed a disappearance of the nystagmus cupulogram response decline. In two other experiments the maximal blood alcohol concentration was as high as 1.12 and 1.39‰ resulting in such intoxication, severe nausea and vomiting, sweating etc., that no reliable recording could be obtained.

In addition to the ten fighter pilots two figure skaters and two female ballet dancers with marked response decline in their nystagmus cupulograms were tested with the same result—the response decline disappearing during alcohol intoxication. Seven of the fighter pilots and the four other subjects had 24 hours after the start of the experiment still not recovered their response decline. However, in all except one this was recovered after about 48 hours.

It must be pointed out that these test subjects in series C all had normal otoneurological findings, with the exception of the cupulogram mentioned. These findings included a normal and symmetrical calorigram. In fact they all had "ideal" recordings when tested calorically.

DISCUSSION AND CONCLUSIONS

In the experimental series A and B there is a discrepancy between the results of the sensation and nystagmus cupulograms. With repeated rotation tests a significance response decline, which is more marked with stronger stimuli, is recorded in the sensation cupulogram. This is best seen when using the first cupulogram as a reference. The control (placebo) test in series B confirms this observation from series A. Series A shows no response decline in the nystagmus cupulogram but in series B with the more frequently repeated stimulations, such a tendency is observed.

On comparing the sensation and nystagmus cupulograms, combined with drug administration in series B there is a great discrepancy between the results, which is seen particularly from the blind tests.

These differences in response to the same peripheral stimuli make it more reasonable to assume that central processes are responsible for the decrease in the post rotatory sensation. The term habituation could thus be justified. Smith (1941), Zelenka (1960) and others have claimed, from physiological and/or pharmacological experiments, that habituation depends on the function of the cortex and Sharplers & Jasper (1956) and Gernandt & Gilman (1960) the function of the brain stem. Hagbarth (1960) stated that the habituation processes affect in a specific fashion the responses to the particular stimuli that have been repeated. This phenomenon could more easily be explained by a specific feed back system acting upon the

sensory neurons. The efferent system in the cupulae described by Wersäll (1956) and Engström & Wersäll (1958) could provide the neuroanatomical basis for thi hypothesis. Studies on the fighter pilots with a marked response decline to rotatory stimuli (Aschan, 1954) also appeared to show that a central mechanism could be the cause of the habituation. The reason for this conclusion was that despite the response decline to rotatory stimuli the caloric reaction in these test subjects was absolute by normal, and this observation was reconfirmed in those included in series C.

Fearing & Mowrer (1934) found that a response decline did not develop under light anaesthesia. Hood & Pfaltz (1954) made the same observation on rabbits. These experimental findings seem to have some relevance to the present findings in series C. In these test subjects a moderate alcohol intoxication with a maximal blood alcohol concentration of up to 1.0‰ made the response decline in the nystagmus cupulogram disappear completely To describe the alcohol intoxication as a light anaesthesia and a depres ion of the central nervous system seems justifiable Goodman & Gilman (1955) also classify ethyl alcohol as a basal anaesthetic in their textbook Aschan, Grant & Ekvall (1964) have shown in rabbits that alcohol int xication has a depressor effect on nystagmus, inhibiting both the type due to central, brain stem or cerebellar lesions and the nystagmus in duced by central stimulation over permanent implanted electrodes in these regions. On the other hand positional alcoholic nystagmus results from the int ication as in the fighter pilots. From the animal experiments mentioned it may be concluded that the disappearance of the rotatory habituation is due to an alcohol effect above the brain stem level The results from series C can thus be regarded as a complement to and concordant with the observation of Fearing & Mowrer (1934) and Hood & Pfaltz (1954) The fact that higher degrees of intoxication did not give acceptable record fits into the picture—the anaesthesia" was too deep

With regard to the experiments with cupulograms bef re and after drug admini tration, it seems apparent that no conclusions can be drawn from the sensation cupulograms. The control placebo test clearly shows the error in olved For the nystagmu cupulograms it might seem that reliable result were obt ined for those drugs showing a "significant" reduction n the ny tagmic response On the other hand, th more intense stimula tion in series B also resulted in a tendency to habituation for the nystagmic response n t present in series A Using the second nystagmu cupulogram in the blind test as a reference thus correcting for the habituation results in that only one drug, no. 3 showed a reduction of the nystagmic response that could be attributed to the drug administration

Compared to th result of de Wit (1953) it must be said that the sensation cupulogram gives no information about the inhibitory effect of the drugs tested on post rotatory vestibular sensations. Criticism is also due in connection with nystagmu cupulograms because here, with repeated tests, habituation influences the results du to estibular habituation On

the other hand there are very few means of testing antinausea drugs experimentally in man with a possibility of obtaining objective records of the results. In these experiments only one drug, viz. methylscopolamini nitras 0.5 mg, pentymalum 50 mg (Veryl® Pharmacia) reduced the post rotatory nystagmic response significantly

RESUME

La cupulométrie au cours d'épreuves giratoires répétées à cinq reprises espacées chacune de deux jours a montré un amortissement de réaction Celui-ci est seulement significatif dans les cupulogrammes de sensation. L'amortissement de la réponse est d'autant plus marqué que l'intensité des stimulations giratoires a été plus forte

Dans une seconde série on a comparé les cupulogrammes enregistrés avant, puis deux heures après la prise de quatre variétés de drogues antinauséeuses d'une part d'un placebo d'autre part L'analyse statistique montre une telle action du placebo sur le cupulogramme de sensation que l'on ne peut tirer de conclusion valable quant aux effets thérapeutiques des drogues sur la sensation post giratoire Les résultats sont plus satisfaisants sur les cupulogrammes de nystagmus, mais l'amortissement de réaction lié à l'augmentation du nombre de stimulations introduit une cause d'erreur dans l'interprétation des résultats. Un seul produit consistant en l'association de 0,5 mg de nitrate de méthylscopolamine et de 50 mg de pentymalum (Veryl® Pharmacia) a diminué de façon significative la réponse nystagmique post-giratoire

Dans une troisième série on a étudié les réactions des pilotes de chasse à l'absorption d'alcool L'amortissement de réaction consécutif à l'entraînement de ces sujets disparait au cours d'intoxications alcooliques modérées. Les réponses normales et symétriques aux épreuves caloriques préalables à l'absorption d'alcool font exclure la possibilité d'une origine labyrinthique aux amortissements de réaction

D'après les données expérimentales et les résultats de cette étude il semble que l'alcool se comporte comme un anesthésique léger qui éliminerait la réponse amortie Il parait donc justifié de la considérer comme une adaptation Elle doit être d'origine centrale et sa disparition est probablement due à un effet dépresseur de l'alcool au-dessus du tronc cérébral et de l'étage cérébelleux

ZUSAMMENFASSUNG

Wiederholte Rotationsversuche wurden mit Cupulometrie durchgeführt (ein Versuch 5mal jeden zweiten Tag) wobei eine signifikativ verminderte Antwort nur im Sensationscupulogramm erzielt wurde Die Verminderung war um so kräftiger je kraftiger der Rotationsstimulus war

In einer zweiten Versuchsreihe wurden 20 Versuchspersonen mittels Cupulogramms vor und zwei Stunden nach Einnahme von vier verschiedenen Antinausea mitteln und einem Placeboversuch getestet Sämtliche Versuchspersonen wurden mit allen fünf Möglichkeiten getestet unter systematischer Abwechslung und mit bis 3 Tagen zwischen jedem Versuch Die statistische Bearbeitung des Sensa-

tionscupulogramms vor und nach der Behandlung zeigt beim Placeboversuch einen solchen „Effekt" dass jeder Schlussatz hinsichtlich eines eventuellen therapeutischen Eff kies ausgeschlossen war Beim Nystagmuscupulogramm war das Ergebnis etwas besser Jedoch ergab das häufigere Rotieren in dieser Ver suchsreihe eine Andeutung über eine gesenkte Antwort selbst beim Nystagmus-cupulogramm dieser Reihe Daraus lässt sich schliessen, dass nur ein Mittel, nämlich Methylscopolamini nitras 0,5 mg und Pentymalum 50 mg (Veryl® Pharmacia) eine signifikant Reduktion der postrotatorischen Nystagmusant wort ergab.

In einer dritten Versuchsreihe wurden erf hrene Jagdfli ger mit ausgespro-chen schwacher Antwort auf Rotationsteste, vor und nach Alkoholgenuss mit t h Cupulometrie untersucht, wobei die maximalen Blutalkoholkonzentrationen di 1,0-pro-mille-Grenze errei hten. Während der Berauschung verschwand die durch den Beruf erworbene Gewöhnung und völlige Restitution fand erst 24 bis 48 Stunden nach Alkoholgen ss statt. Andere Untersuchungen und dieses Unter suchungsergebnis lassen vermuten dass Alkohol als ein leichtes Anästhetikum wirkt und die berufsmässig erworbene Gewöhnung der Jagdflieger an rotato-r sche Reizung eliminiert. Diese Gewöhnung muss zentral bedingt sein und ist wahrscheinli h bedingt durch einen Depressoreffekt von Alkohol berhalb des Gehirnstammes und des Kleinhirnni eaus.

REFERENCES

Aschan, G 1954 Responses t rotatory stimuli i fighter pilots. *Act Otolaryng* (Stockh.) Suppl. *116* 24

— 1955 The caloric test. *A 1 Soc Med. Upsal.*, *60* 99

— 1958 Different types of lcohol nystagmus. *Act Otolaryng* (Stockh.) Suppl. *140* 69

Aschan, G., Bergstedt M nd Goldberg, L., 1964 Positional lcohol nystagmus i patient with unilateral nd bilateral labyrinthine destructions. *Confin. Neurol* 24 80

Aschan, G Bergstedt M Goldberg, L., nd La rell, L., 1956 Positional ystagmus in man during nd fter lcohol int ication. *Quart J Stud. Alcohol* *17* 381

Aschan G Bergstedt M nd S ahl, J 1956 Nystagmography recording f nystag mus i li ical neuro-otological examinations *Act Otolaryng* (Stockh.) S ppl. *129*

Aschan, C Grant G nd Ek all, L., 1964 Ny tagmus following stim lation i central estibula pathw y sing permanent implanted electrodes. *Act Otolaryng* (Stockh.) S ppl *192* 63

Aschan, C Kylén, C O., S ahl, J nd Wersäll, R 1953 The rot tion test. Cupulo-metric data from 320 normals. *Act Otolaryng* (Stockh) 42 431

Aschan, G Petterson, C., nd Ostermorg, L., 1953 A rotatory cabinet with electric dri g uni *Acta Ol laryng* (Stockh.) 43, 22.

Di ler D., 1957 *Phy iologie und Patholog i des Bogengangsapparates beim M nschen* Deuticke Le pzig & Wien.

Dodge, R 1923 Habit tion t rotation. *J Exper Psychol* 6 1

Eg ston, \ A J Oscar J J nd Johnson, L. B. W 1949 The t rning test with small regulable t m li *J Laryng* *63*, 399.

Eng röm, H. nd W rsäll, J 1958 The ltrastructural organization of the orga of Corti nd the estibul sensory epithelia. *Exper Cell R* Suppl. 5 460.

Frantzel, F nd Monnier, O. H., 1931 The effect f general anesthesia pon the perimental f reduction of estibular ystagm s. *J G n. Psych l.*, *11* 133.

FISCHER, M H 1928 Die Regulation funktion des menschlichen Labyrinthes und die Zusammenhänge mit verwandten Funktionen *Ergebn Physiol* 27 209
GERNANDT B E, and GILMAN S 1960: Vestibular and propriospinal interactions and protracted spinal inhibition by brain tem acti tion *J N u phy iol* 23 269
GOODM N L, S nd GILMAN A 1955 *Th Pharmac logical Basi f Th rapeutics.* The Macmill n Company New York.
HAGBARTH, K E, 1960 Centrifug l mechanism of sen ory control *Ergebn Biologie* 22, 47 (Ed. H Autrum) Springer Verlag Berlin
HALLPIKE, C. S nd HOOD, J D 1953 Fatigue and daptation of the amp lar mechanism in the human hori ntal semicircular canal *Proc Roy Soc* (Biol) *141* 542.
H MBERGER, C. A and HYDÉ H 1949 Production of nucleoproteins in the vestibular ganglion *Acta Ot laryng* (Stockh) Suppl 75 63
HOLSOPPLE J A 1924: An e planati n fo unequal reduction in po t-rot tion nystagmus. *J Comp I hysi l P ych l* 4 185
HOOD, J D and PFALTZ, C. R 1954 Observati ns upon the effect of repeated stimula ti n upon rotati nal and cal ric nystagmus *J Physiol., 124* 130
MITTERMAIER, R 1952 Über die Beeinflussung des e perimentell ausgelöst n Nystagmus durch wiederh lte Reizungen *Z Laryng Rhinol Otol 1* 360.
SHARPLESS, S and J SPER, H 1956: Habituation f the arousal reacti n. *Brain, 79* 655.
SMITH K. U 1941 The effect of partial and complete decorticatio upon the e tinction of optic nystagmus. *J Gen Psych l 25* 3
WERSÄLL, J 1956 Studi on the tructure and innerv tion of the sensory pithelium of the cri tal ampullares in the guinea pig *Acta Ot laryng* (Stockh) Suppl *126*
DE WIT C 1953 Sea ickness. *Acta Ot laryng* (Stockh) S ppl *108*
ZELENKA J 1960: Recherches expérimentales u l influence d système nerveux central su l' pparell vestibulaire à l aid de l'hydrat de chloral *R v Laryng* (Bord.) *81* 244

D pt of Otolaryngology Ak demiska Sjukhus t Upps la Sweden

Received Decemb 9 1966

Act oto-laryngologica 64 107 118 (1967)

NEGATIVE POTENTIAL IN SCALA MEDIA DURING EARLY STAGE OF ANOXIA

T Konishi Elizabeth Kelsey and G T Singleton
Gainesville Fla U.S A

From the Division of Otolaryngology the University of Florida Coll ge of Medicin Gainesville

Negat potential in scala media during early anoxi was recorded in both normal and kanamy in-treated guinea pigs. In kanamycin-treated nimals, in which the cochlear microphonic was remarkably depressed declin f the d potential was slower and its negative value smaller than I non-treated imals. After prolonged sphyxia, amplitud of post mortem cochlear microphonic was increased by n anodal current The polarity of the negati e pot ntial could be changed by application of positive pressure to scala vestibuli, whereby the postmortem cochlear microphonic was enhanced However neither application of pressure nor current produced any enhancement of cochlear microphoni fter the negati potential in scala media reached zero These data indicate that the n gati e potential in the scala media of the sphyxiated cochlea is ascribed t depolarization f the ormally polari ed hair-bearing ends f the hair cell d that stria vascularis is se itive while the hai -bearing ends f the hair cells are insen itive t o ygen deprivatio

Previous tudies have demonstrated that the endocochlear potential (EP) is sensitive to oxygen deprivation and, if the oxygen supply is permanently closed off FP falls temporarily below zero to a negative value of as much a 40 mV before gradually returning to a final zero level post mortem (v Békésy 1952 Ci selsson, 1950 Konishi, Butler & Fernández, 1961 Rice & Shinabarger 1961) When th blood supply to the cochlea is only temporarily obstructed, EP returns to its original or upernormal value shortly after the restoration of blood flow The origin of this negative potential in the scala media during anoxia is still obscure although it has been discussed by several investigators (v Békésy 1952 Konishi, Butler & Fernández, 1961 Rice 1961 Honrubia, Johnstone & Butler 1965 Johnstone, 1965)

The experiments to be reported here were designed to investigate the nature of the reversal in the polarity of the dc potential in the scala media during anoxia. For this purpose changes in EP during an xia were tudied in kanamycin-treated guinea pigs in which the organ of Corti had largely degenerated. Applications of external direct current r of hydrostatic pres-

Thi research was supported in large part by grant NB 03414 from the N tional In titut of Health.

sure to the cochlea were chosen to modify the condition of the hair-bearing ends of the haircells in the organ of Corti These agents are known to cause reversible changes both in EP and the cochlear microphonic (CM) in the normal state (Tasaki & Fernández, 1952 Tasaki Davis & Eldredge, 1954)

METHODS

Experiments were carried out in normal guinea pigs and in animals which had been given kanamycin subcutaneously in doses of 800 mg per kg body weight daily for 5 to 7 days and kept 4 to 6 weeks thereafter In order to measure EP a small fenestra was made over the spiral ligament of the basal turn so that a glass pipette electrode mounted on a micromanipulator could be inserted into the scala media EP was measured by means of an electrometer Permanent records of the dc potential were obtained by delivering the output of the electrometer to an oscillograph

When recording CM summating potential (SP) and action potential of the auditory nerve (AP) the differential electrode technique was employed (Tasaki & Fernández, 1952) A chloride-coated silver wire placed on the neck muscles served as the reference electrode After suitable amplification, CM SP and AP were displayed on an oscilloscope and photographed

The sound stimuli employed were tone bursts of from 4000 to 10 000 cps. The sound was delivered to the animal through a speculum fixed in the external auditory canal The connection between the speculum and an earphone was made with a polyethylene tube

A direct current for polarization of the cochlear partition was applied through fine glass pipettes inserted into the scala media and the scala tympani The scala tympani pipette had a tip diameter of approximately 150 microns and was filled with Ringer's solution The method of current application has been described by Tasaki Davis & Eldredge 1954

In order to apply hydrostatic pressure to the scala vestibuli a small hole was made in the scala vestibuli of the basal turn A similar hole was made in the scala tympani of the basal turn for application of hydrostatic pressure to the scala tympani An outlet was made by providing another small hole in the apex An injection pipette whose tip diameter ranged from 50 to 100 microns, was connected by a polyethylene tube to a syringe and the whole system was filled with Ringer's solution Hydrostatic pressure was applied to the scala vestibuli or scala tympani by injecting Ringer's solution from this syringe The degree of the pressure was controlled by adjusting the pressure applied to the syringe plunger The duration of the pressure was 30 to 60 seconds

Testing procedures

Following surgery the guinea pig was injected with a paralyzing dose (0 1 cc) of *d* tubocurarine chloride intravenously and connected to an

artificial respirator The head was rigidly mounted on a specially designed metal holder After CM SP and AP elicited by the tone bursts were recorded, the glass pipette electrode was inserted into the scala media through the spiral ligament to measure EP At this time photographs of cochlear potentials in response to acoustic stimuli, as well as EP readings were taken. Next, anoxia was produced either by occluding the anterior inferior cerebellar artery from which the cochlea receives its blood supply (Kimura & Perlman 1958) or a phyxia was induced by simply turning off the artificial respirator Throughout the period of occlusion of the anterior inferi r cerebellar artery absence of the blood flow in the radiating arteries in the fourth turn of the cochlea was monitored periodically Measurements of cochlear potentials were made every 15 seconds immediately after anoxia was induced When the rate of change of the potentials decreased, records were taken less frequently

The effects of polarizing current or hydrostatic pressure on the cochlear pot ntials were tested when the negative potential in the scala media reached a maximum and again when it returned to zero level. The external polarizing current was applied to the cochlea through the glass pipette electrode which had been inserted into the scala media. The glass pipette electrode was disconnected from the dc recording circuit and connected to the polarizing circuit The current introduced to the cochlea was limited t 10 t 20 microamperes and it duration to less than 3 seconds in order to void formation of bubbles in the pipette Cochlear potential recordings were made before during and after current application. The dc potential in the scala media was not measured during the current flow

In order to test variations in cochlear potentials caused by pressure change in the perilymphatic space, the injection pipette mounted on a micromanipulator was deeply inserted into the scala vestibuli or scala tympani, and positive pressure was initiated by pressing the syringe plu ger Usually transient positive pressure could be applied to the cochlea for bout one minute. Excess fluid accumulated in the bulla was continuously removed with a small sucti n tube The cochlear potentials in response to sound were photographed every 2.5 seconds and EP was graphically recorded during and after the applicati n of pressure to the perilymphatic space Upon compl letion of the experiment, temporal bones of kanamycin-treated guinea pigs were preserved for histological *examination*.

RESULTS

1 *Changes in FP during anoxia in kanamycin treated guinea pigs*

In eight kanamycin treated guinea pigs, the responses of CM, SP and AP were greatly reduced Table 1 shows the levation of the pseudothreshold of CM in these animals. EP w found to be within normal limit r *greater* than normal. It ranged from 93 to 98 mV with an average f 9 .3 mV When

sure to the cochlea were chosen to modify the condition of the hair bearing ends of the haircells in the organ of Corti These agents are known to cause reversible changes both in EP and the cochlear microphonic (CM) in the normal state (Tasaki & Fernández, 1952 Tasaki Davis & Eldredge 1954)

METHODS

Experiments were carried out in normal guinea pigs and in animals which had been given kanamycin subcutaneously in doses of 800 mg per kg body weight daily for 5 to 7 days and kept 4 to 6 weeks thereafter In order to measure EP a small fenestra was made over the spiral ligament of the basal turn so that a glass pipette electrode mounted on a micromanipulator could be inserted into the scala media. EP was measured by means of an electrometer Permanent records of the dc potential were obtained by delivering the output of the electrometer to an oscillograph

When recording CM summating potential (SP) and action potential of the auditory nerve (AP) the differential electrode technique was employed (Tasaki & Fernández, 1952) A chloride-coated silver wire placed on the neck muscles served as the reference electrode After suitable amplification, CM SP and AP were displayed on an oscilloscope and photographed

The sound stimuli employed were tone bursts of from 4000 to 10 000 cps. The sound was delivered to the animal through a speculum fixed in the external auditory canal The connection between the speculum and an earphone was made with a polyethylene tube.

A direct current for polarization of the cochlear partition was applied through fine glass pipettes inserted into the scala media and the scala tympani The scala tympani pipette had a tip diameter of approximately 150 microns and was filled with Ringer s solution The method of current application has been described by Tasaki Davis & Eldredge 1954

In order to apply hydrostatic pressure to the scala vestibuli a small hole was made in the scala vestibuli of the basal turn A similar hole was made in the scala tympani of the basal turn for application of hydrostatic pressure to the scala tympani An outlet was made by providing another small hole in the apex An injection pipette, whose tip diameter ranged from 50 to 100 microns, was connected by a polyethylene tube to a syringe and the whole system was filled with Ringer s solution Hydrostatic pressure was applied to the scala vestibuli or scala tympani by injecting Ringer s solution from this syringe The degree of the pressure was controlled by adjusting the pressure applied to the syringe plunger The duration of the pressure was 30 to 60 seconds.

Testing procedures

Following surgery the guinea pig was injected with a paralyzing dose (0 1 cc) of d tubocurarine chloride intravenously and connected to an

artificial respirator The head was rigidly mounted on a specially designed metal holder After CM, SP and AP elicited by the tone bursts were recorded, the glass pipette electrode was inserted into the scala media through the spiral ligament to measure EP At this time photographs of cochlear potentials in response to acoustic stimuli, as well as EP readings were taken. Next, anoxia was produced either by occluding the anterior inferior cerebellar artery from which the cochlea receives its blood supply (Kimura & Perlman, 1958) or asphyxia was induced by simply turning off the artificial respirator Throughout the period of occlusion of the anterior inferior cerebellar artery absence of the blood flow in the radiating arteries in the fourth turn of the cochlea was monitored periodically Measurements of cochlear potentials were made every 15 seconds immediately after anoxia was induced. When the rate of change of the potentials decreased, records were taken less frequently

The effects of polarizing current or hydrostatic pressure on the cochlear potentials were tested when the negative potential in the scala media reached a maximum and again when it returned to zero level. The external polarizing current was applied to the cochlea through the glass pipette electrode which had been inserted into the scala media. The glass pipette electrode was disconnected from the dc recording circuit and connected to the polarizing circuit The current introduced to the cochlea was limited to 10 to 20 microamperes and its duration to less than 3 seconds in order t void formation of bubbles in the pipette Cochlear potential recordings were made before during and after current application The dc potential in the scala media was not measured during the current flow

In order to test ariations in cochlear potentials caused by pressure change in the perilymphatic space the injection pipette mounted on a micromanipulator was deeply inserted into the scala vestibuli or scala tympani, and positive pressure wa initiated by pressing the syringe plunger Usually transient positive pressure could be applied to the cochlea f r about n minute Excess fluid accumulated in the bulla was continuously removed with a mall suction tube The cochlear potentials in response to sound were photographed every 2.5 seconds and EP was graphically recorded during and after the application of pressure to the perilymphati space Upon completion of the experiment, temporal bones of kanamycin treated guinea pigs were preserved for histological examination.

RESULTS

1 *Changes in EP during anoxia in kanamycin-treated guinea pigs*

In ight k namycin-treated guinea pigs, the responses of CM SP and AP were greatly reduced Table 1 shows the elevation of the pseudothreshold of CM in these animals. EP was found to be within normal limits or greater than normal. It ranged from 93 to 98 mV with an average of 95.3 mV When

TABLE 1 *Dependence of negative potential in anoxic scala media on depression of CM in kanamycin treated guinea pigs*

Animal No	Cochlear microphonic pseudothreshold in dB[a] 500 cps	1000 cps	2000 cps	4000 cps	8000 cps	EP (mV)	Survival time of EP	Maximum value (mV) of negative potential in anoxic scala media
Non treated	0	0	0	0	0	88	30.5 sec[b]	−4.5[b]
C-185	41	50	r	60	51	9	25 min	−3
C 186	35	37	35	41	30	95	17 min	−8
C-187	40	75	78	65	43	97	37.5 min	−4
C-198	35	30	35	30	20	98	20 min[c]	−9
C 199	40	38	43	48	43	91	8.5 min	−15
C-200	55	52	50	48	40	—	—	—
C-201	6	50	62	60	50	93	18 min	−7
C-202	23	41	53	60	35	—	—	—

[a] Reference taken as average CM pseudothreshold of 10 non treated guinea pigs.
[b] Quoted from Konishi et al 1961 *J Acoust Soc Amer* 33 349
[c] *Partial recovery of blood flow during occlusion of anterior inferior cerebellar artery*

the anterior inferior cerebellar artery was occluded in the animals, the slope of the decline in EP was extremely slow and EP reached zero

The survival time of EP the elapsed time between oxygen deprivation and disappearance of the positive potential ranged from 8.5 min to 37.5 min as shown in Table 1 Except in one case in which partial recovery of the blood flow was observed during the occlusion of the artery the survival time of EP seemed to be related to the degree of depression of CM In other words, it was longer in guinea pigs with severely depressed CM than in those moderately poisoned EP upon reaching zero continued to decline gradually exhibiting a change in polarity This change in polarity took place when the reference electrode was placed either on the neck muscles or in the scala tympani of the basal turn The magnitude of the negative potential in the scala media was less than 10 mV except in one moderately poisoned animal in which the survival time of EP and maximum negative potential were 8 minutes 30 seconds and −15 mV respectively As illustrated in Table 1 the more severely CM was depressed, the less the negative potential obtained in the scala media during anoxia. With occlusion of 50 minutes duration it was observed that this negative potential after reaching maximum value began to return toward zero The rate at which this potential decreased was slow

In 3 animals, application of the occlusion probe damaged the artery and blood flow did not return when the probe was withdrawn In the other 3 cases in which the return of blood flow was observed after release of the probe prompt recovery of EP was noted The change of the polarity occurred within 15 seconds after the recovery of blood flow None of these

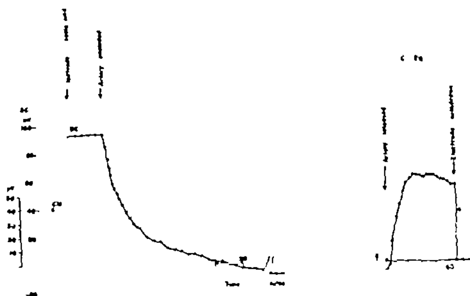


FIG. 1. A example of beha ior f CM and EP i kanamycl -treated guinea pig when occl sion of the nt rior i ferior cerebella rtery was in effect for 51 minutes. Stimulu 6800 cps tone burst t pproximately 35 dB bove CM pseudothreshold.

animals showed complete recovery but EP reached a maximum within 4 to 6 minutes after return of the blood flow and then EP gradually de creased. Fig 1 shows a typical example of changes in dc potential and CM in a kanamycin-treated guinea pig during temporary occlusion of the ant rior inferior cerebellar artery

2. Effe t of polarizing current on postmortem cochlear microphonic

When the polarizing electrode in the scala media of the basal turn was connected to the source of the direct current and that in the scala tympani to the sink and approximately 10 to 20 microamperes were introduced to the cochlea, there was always an increase of the amplitude of CM and the negative SP which was recorded with the differential electrodes in the basal turn. A current flowing in the reverse direction decreased CM. These changes were reversible. When the negative potential reached a maximum value after the animal's death, the same amount of the current was again introduced to the cochlea. CM was greatly enhanced by this polarizing cur rent which made the scala media more positive Usually the negative SP was visible while introducing the current For a few seconds after the current was turned off the postmortem CM was slightly depressed and then returned to the original magnitude No reversal of phase of CM was obtained by the introduction of the external dc current, regardless of the polarity of the dc potential in the scala media. With the source of current

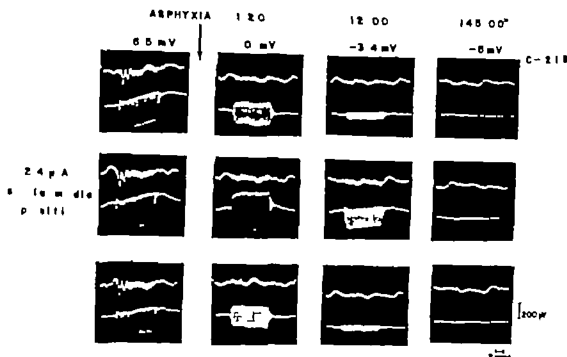
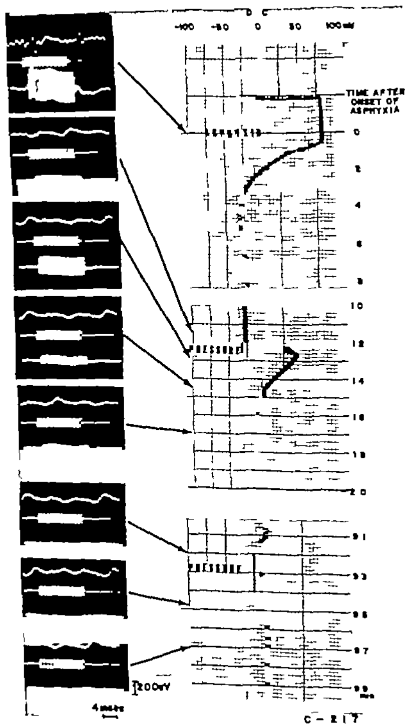


Fig. 2 Changes in cochlea potentials induced by a polarizi g current fl wi g from scala media t scala t mpani in n rmal and a phyxi ted states Numbers in the uppermost line indicate time l psed between onset of asph yxia a d applicati n of the current th se below sh w EP in mV bef re the current w s sent t the cochlea Photographs in the upper middl and lower rows w re tak n bef re during nd aft r the application of current re pectively In ea h photograph the upper tra ing sh ws AP if present, and lower tra ing CM Stimulus, 4000 ps t ne burst at appro imat ly 40 dB above CM threshold

connected to the scala media and the sink to the scala tympani, AP was not enhanced after its disappearance in the asphyxic period.

Polarizing current was again introduced to the cochlea when no dc potential was present in the scala media Usually it took two or three hours when the animal was kept at room temperature (approx 18 C) No effect of the external current on the postmortem CM was ever observed The changes of the postmortem CM in response to the polarizing current became less as anoxia or asphyxia progressed. Fig 2 is an example of the results showing the effect of the polarizing current on the postmortem CM and AP

Fig 3 A ompo it of ev nt occurring du ing nd fte 40 sec pplicatio f hydro- t tic press re t scala estibuli in n phyxi ted guinea pig Photographs f the ochlear respo ses to a 7000 cps t ne burst t pproxim t ly 40 dB bo CM thresh old are sh wn in th l ft colum F ach ph t graph, th t p tra ng shows AP the middle traci g stimulu and th bott m tra i g CM a d SP The right column h ws chang in the d pot nti l in the sc la medi relative t the neck As seen ne th t p of the column, a positi potenti l i regi t red when the lect od wa inserted int the scala media.


D C
-100 -50 0 50 100 mV
TIME AFTER ONSET OF ASPHYXIA
0
2
4
6
8
10
12
14
16
18
20
21
23
25
27
29 min
PRESSURE
PRESSURE
200 μV
4 msec
C - 2 1 7

3 Effect of hydrostatic pressure on cochlear potential in asphyxiated guinea pigs

Rapid filling of the scala vestibuli with Ringer's solution was performed 7 to 10 minutes after the negative potential in the scala media had leveled off and CM reached the postmortem level. Rate of perfusion was found to be critical. However, we could not exert close control over the perfusion rate and thus the hydrostatic pressure applied to the cochlear fluid varied between cases.

Hydrostatic pressure applied to the scala vestibuli during asphyxia caused a decrease of the negativity of the dc potential in the scala media as shown in Fig. 3. When the pressure was great enough, the polarity of the dc potential exceeded 50 mV or more in some cases. Changes in magnitude of CM were not consistent during the application of pressure, even though great care was taken to remove excess fluid accumulated in the auditory bulla by suction. Strikingly, a definite increase of CM was observed immediately after release of the pressure. The negative SP appeared after the application of pressure to the scala vestibuli. However, in none of our cases could AP be observed during this period, in spite of the enhancement of CM and SP. When the perfusion was stopped, the dc potential in the scala media gradually reached its original value after a certain time lapse. Both CM and SP gradually decreased in magnitude and were almost invisible on the oscilloscope. In a few cases, the hydrostatic pressure was applied to the scala vestibuli after the negative dc potential in the scala media reached zero. No pressure effect on the dc potential in the scala media and the postmortem CM was observed in those cases as shown in Fig. 3.

Application of hydrostatic pressure to the scala tympani with Ringer's solution during anoxia had an opposite effect on the negative dc potential in the scala media. It caused an increase of its negativity. After the release of pressure, the dc potential gradually returned to the original value. CM at the postmortem level was further depressed by this pressure application. In general, the magnitude of the dc potential changes in response to hydrostatic pressure applied to either scala vestibuli or scala tympani became less with each successive pressure application. Also, its effect became less as anoxia or asphyxia was prolonged.

DISCUSSION

The methods used in these experiments are similar to those in our previous report concerning the effect of anoxia on cochlear potentials in normal guinea pigs (Konishi, Butler & Fernández, 1961). As shown in Table 1, the absolute magnitude of the negative potential in the scala media during anoxia was much smaller in kanamycin treated guinea pigs than in

normal, even though the initial EP was normal or greater than normal. It is well established by accumulated data (Hawkins, 1959 Ward & Fernández, 1961 Hawkins & Engström, 1963) that in kanamycin poisoned guinea pigs, the stria vascularis is intact, although the hair cells of the organ of Corti have seriously degenerated. Hawkins *et al* (1963) reported that one of the earliest changes observed in kanamycin treated guinea pigs is a disturbance of the orderly W pattern of the stereocilia of the outer hair cells in the basal coil Therefore it is reasonable to assume that difference in decline of EP during the anoxic period between normal and kanamycin treated guinea pigs is the result of the activity of the hair cells of the organ of Corti The potential recorded by the microelectrode in the anoxic scala media is the algebraic sum of two independent potential changes (1) EP which is generated by the stria vascularis (Tasaki & Spyropoulous, 1959) is depressed and reaches zero as anoxia progresses and (2) a negative potential generated by depolarization of the hair cells of the organ of Corti. The fact that CM and especially SP were observed in response to intense sound in kanamycin treated guinea pigs used in our experiments indicates that the hair cells of the organ of Corti have not completely degenerated in the basal turn. This accounts for the smaller than normal negative potential in the scala media as anoxia progressed in these kanamycin-treated animals. Our proposed idea can also be supported by our finding that the higher the pseudothreshold of CM in kanamycin-poisoned animals, the smaller the negative potential in the scala media and the slower its decline during the anoxic period. Theoretically in animals with organ of Corti degeneration the decline of th dc potential during anoxia should represent solely the depression of EP and a negative potential generated by depolarization of hair cells during this period would be shown by the difference of the dc potential in the scala media between normal animals and those without organ of Corti. In order to account for the very rapid decline of EP to −47 mV in normal animals during anoxia (Konishi, Butler & Fernández, 1961) the negative dc potential produced by the hair cells must attain a maximum value a much as −80 mV *within 1 minute after* the occlusion of the anterior inferior cerebellar artery and then gradually decrease until it reaches zero two or three hours after the *onset of* anoxia. The anoxic depolarization in motoneurons has been discussed by several investigators (v Harreveld & Biersteker 1948 1964 Kolmodin & Skoglund, 1959 Nelson & Frank 1959 Eccles, Løyning & Oshima, 1966)

Our assumption concerning the mechanism of the asphyxiated hair cell a tion in terms of membrane potential is further justified by our data on effects of either pressure r external current on the postmortem CM The hair-bearing ends of the hai cells in the organ of Corti are exposed to anodal polarization by the presence of EP Tasaki & Fernández (19 2) reported that when this polarization was increased by an external source of current, amplitudes of both CM and AP were increased. A decrease in the polarization at the hair-bearing ends of the hair cells depresses the

amplitude of CM and AP The ability of anodal current to increase the polarization of the membrane of the anoxic nerve was demonstrated by Lorente de Nó (1947) Our data clearly show that the direction of amplitude changes in postmortem CM was the same as that observed in CM in normal status, when the external polarizing current was introduced in the cochlea We interpret these data to mean that the application of an external dc current from the scala media to scala tympani results in the repolarization of the hair bearing ends of the hair cells which have been depolarized during asphyxia

Honrubia Johnstone & Butler (1965) reported that EP CM and SP could be restored to a relatively high level during anoxia of the scala vestibuli was perfused with either Ringer s or KCl solution On the basis of these experiments, they proposed that the negative potential in the scala media during asphyxia is due to accumulation of toxic agents during anaerobic metabolism We reconfirmed their data, but interpreted it differently As already pointed out the absolute changes in the dc potential produced by the perfusion were closely related to the pressure applied to the scala vestibuli Secondly the magnitude of the changes in the negative dc potential during the perfusion became less pronounced as anoxia was continued and finally no change in the dc potential was obtained in the scala media when the negative potential reached zero In addition to this, as extremely strong positive pressure was repeatedly applied to the scala vestibuli during anoxia the responsiveness of the negative potential became less sensitive and finally no restoration of CM could be observed These facts strongly indicate that the primary function of the perfusion of the scala vestibuli is not to remove anaerobic metabolites that accumulate in the anoxic cochlea as suggested by Honrubia Johnstone & Butler (1965) but to displace the basilar membrane toward the scala tympani Tasaki, Davis & Eldredge (1954) clearly demonstrated that in the normal state the dc generators on the basilar membrane produced a positive potential when the basilar membrane is displaced toward the scala vestibuli, yet the stria vascularis is not affected This is always the case regardless of whether the cochlea is anoxic or not (Butler & Honrubia, 1963) Therefore the diplacement of the basilar membrane toward the scala tympani causes the repolarization of the hair bearing ends of the hair cells which have been depolarized by oxygen deprivation, and consequently postmortem CM is enhanced by restoration of a large potential drop across the hair bearing ends of the hair cells. Therefore reversal of the polarity of the potential in the scala media does not mean restoration of EP

Our findings that postmortem CM can be enhanced by introduction of either external anodal current or displacement of the basilar membrane indicate that while the stria vascularis is sensitive to oxygen deprivation the hair bearing ends of the hair cells retain their ability to be activated when repolarized This is also confirmed by us (paper in preparation) in that the effective resistance of the cochlear partition between the scala

media and scala tympani does not show appreciable changes in the anoxic period until disappearance of the negative dc potential in the scala media

CONCLUSIONS

(1) In anoxic kanamycin-poisoned guinea pigs, there is a strikingly slower decline of the dc potential in the scala media. Maximum negative value is also smaller than that in normal animals.

(2) A dc current flowing to the anoxic cochlear partition from the scala media to the scala tympani enhances the postmortem CM.

(3) An increase of hydrostatic pressure in the scala vestibuli results in an enhancement of the postmortem CM in anoxic animals.

(4) From these findings, we conclude that the negative potential in the scala media during anoxia can be attributed to the depolarization of the hair-bearing ends of the hair cells. The primary function of anodal polarization is to repolarize the hair-bearing ends and consequently the postmortem CM is enhanced. Mechanical displacement of the basilar membrane toward the scala tympani result in repolarization of the hair bearing ends of the hair cells.

(5) The hair bearing ends of hair cells retain their ability to be activated when repolarized, while the stria vascularis is sensitive to oxygen deprivation.

ZUSAMMENFASSUNG

Das negative Potential in der Scala media während der Frühstadien der An xie wurde in normalen und kanamycinvorbehand lten Meerschweinchen *registriert*. *Bei* kanamycinbeha delten Tieren, in denen das cochlear Mikrophon-Potential *weitgehend unterdrückt* wa wa der Abf ll des Gleichstrom-Potentials langsamer und dessen negativer Wert geri ger als bei unbehand lt n Tieren. Nach längerer Asphyxie wurd die Amplitud des post-mortem cochlearen Mikrophon-Potentials durch inen Anode strom erhöht. Die Polarität des egativen Potentials konnt durch positi en Druck *auf* die Scala vestibuli geändert werden wodurch das post-mortem cochleare Microphon Potential *vergrössert* wurde. *Dagegen* resultierten weder die Anw dung von Druck noch von Strom in einer Vergrösserung des cochlearen Mikrophon-*Potentials*, wenn da negative Potential in der *Scala* media auf null gefallen war. Diese Daten zeigen da s d negati e Potential in der Scala media der asphyxierten Cochlea auf einer Depolarisation von normaler weise polarisierten haartragenden Enden der Haarzellen beruht und dass di Stria vasculari gegenüber Anoxie sensiti ist, während die haartragend n Enden der Haarzellen insensiti gegenüber Anoxie ind

REFERENCES

ov Bé ésy G., 1953. Gross localization of the place of origi of the cochl ar microphonics. *J. Acoust. Soc. Amer.* 25, 399.

Butler, R. A., and Hon ma, Y. 1963. Responses of cochlear potential t hanges i hydrostatic pressure. *J. Acoust. Soc. Amer.* 35, 1188.

ECCLES, R. M., LØYNING, Y. and OSHIMA, T., 1966: Effect of hypoxia on the monosynaptic reflex pathway in the cat spinal cord. *J. Neurophysiol. 29*, 315.

GISSELSSON, L., 1955: Neuere Probleme des Cochleareffektes. *Arch. Ohr. Nas. Kehlkopfheilk. 167*, 274.

HAWKINS, J. E., JR. and ENGSTRÖM, H., 1963: Effect of kanamycin on cochlear cytoarchitecture. *Acta Otolaryng.* (Stockh.) Suppl. *188*, 100.

HAWKINS, J. E., JR., 1959: The ototoxicity of kanamycin. *Ann. Otol. 68*, 698.

VAN HARREVELD, A., 1946: Asphyxial depolarization in the spinal cord. *Amer. J. Physiol. 147*, 669.

VAN HARREVELD, A. and BIERSTEKER, P. A., 1964: Acute asphyxiation of the spinal cord and of other sections of the nervous system. *Amer. J. Physiol. 206*, 8.

HONRUBIA, V., JOHNSTONE, B. M. and BUTLER, R. A., 1965: Maintenance of cochlear potentials during asphyxia. *Acta Otolaryng.* (Stockh.) *60*, 2.

JOHNSTONE, B. M., 1965: The relation between endolymph and the endocochlear potential during anoxia. *Acta Otolaryng.* (Stockh.) *60*, 2.

KIMURA, R. and PERLMAN, H. B., 1958: Arterial obstruction of the labyrinth. *Ann. Otol. 67*, 5.

KOLMODIN, G. M. and SKOGLUND, C. R., 1959: Influence of asphyxia on membrane potential level and action potentials of spinal moto- and interneurons. *Acta Physiol. Scand. 45*, 1.

KONISHI, T., BUTLER, R. A. and FERNÁNDEZ, C., 1961: Effect of anoxia on cochlear potentials. *J. Acoust. Soc. Amer. 33*, 349.

LORENTE DE NÓ, R., 1947: A study of nerve physiology. *Studies from The Rockefeller Institute for Medical Research*, New York, 132.

NELSON, P. G. and FRANK, K., 1959: Effect of anoxia and asphyxia on cat spinal motoneurons. *Physiologist 2*, 88.

RICE, E. A., 1961: Origin of Corti's negative intracellular polarization. *U.S.A.F. Wright Air Development Division Technical Report 60*, 778.

RICE, E. A. and SHINABARGER, E. W., 1961: Studies on the endolymphatic dc potential of the guinea pig's cochlea. *J. Acoust. Soc. Amer. 33*, 922.

TASAKI, I., DAVIS, H. and ELDREDGE, D. H., 1954: Exploration of cochlear potentials in guinea pig with microelectrode. *J. Acoust. Soc. Amer. 26*, 765.

TASAKI, I. and FERNÁNDEZ, C., 1952: Modification of cochlear microphonics and action potentials by KCl solution and by direct currents. *J. Neurophysiol. 15*, 497.

TASAKI, I. and SPYROPOULOS, C. S., 1959: Stria vascularis as source of endocochlear potential. *J. Neurophysiol. 22*, 149.

WARD, P. H. and FERNÁNDEZ, C., 1961: The ototoxicity of kanamycin in guinea pigs. *Ann. Otol. 70*, 132.

Div. of Otolaryngology, the University of Florida College of Medicine, Gainesville, Fla., U.S.A.

Received September 20, 1966

Acta oto-laryngologica 64 119–121 (1967)

DIE NORMALE VESTIBULARE ERREGBARKEIT VON AFFEN (MACACUS RHESUS) IM DREHVERSUCH BEI ELEKTRONYSTAGMOGRAPHISCHER ABLEITUNG[1]

G LANGE
Freiburg i Br Deutschland

Aus der Hals-Nasen-Ohrenklinik der Universität (Direktor Prof Dr F Zöllner) Freiburg i. Br

Der durchschnittli he estibuläre Schwellenwert des wachen Rhesusaffen liegt unterhalb einer Beschleunigung von 0,37 sec² (bei Ausscheidung offensichtlich ermüdeter Tiere unter 0,2° sec²) Dauer (40) Frequen (33 Schläge) Amplitude (3) und Winkelgeschwindigkeit der langsamen Phasen (76,5 /sec) lassen sich leicht bestimmen Die Augenbewegungen wurden el ktronystagmographisch über Platin Einsteck lektroden registriert.

EINLEITUNG

Im Rahmen einer Untersuchung über Möglichkeiten der Toxizitätsmin derung ototoxischer Pharmaka war es notwendig, die normalen vestibulä ren Erregbarkeitsverhältnisse bei Rhesusaffen zu bestimmen. Wir haben uns dabei vorläufig auf Drehreize beschränkt und zusätzlich Spontan nystagmus sowie optokinetischen Nystagmus registriert. Die Bestimmung der normalen vestibulären Schwelle mit Hilfe der Nystagmographie ist bei Tieren unseres Wissens noch nicht erfolgt Frühere Untersucher (Buys & Rijlant, 1939 Mowrer 1935) suchten die Schwellen für Kopfnicknystag mus bei Tauben und Froschen und fanden sie bei einer Beschleunigung von 0,8 sec² Ek Jongkees & Klijn (1959) konnten mit Hilfe der Myographie schon bei 0 01 sec² regelmäßige vestibuläre Reaktionen registrieren Ter Braak (1937) sah solche bei Kaninchen ab 0,25 sec²

Von der Empfindlichkeit der Registriermethode hängt auch bei kli nischer Untersuchung das Ergebnis vestibulärer Prüfungen ab. Montandon & Ru sbach (1955) fanden die vestibuläre Schwelle für Drehreize um 0,8 sec² Decher (1962) in einer päteren Untersuchung um 0 4 sec² Diese Untersucher registrierten elektronystagmographisch und griffen die Potentialänderungen mit Hilfe von Klebeelektroden ab Die empfindlichere Photoelektronystagmographie ließ Pfaltz (1960) Normalwerte um 0,25 sec² finden

[1] Dr H Lange sen. zum 70. Geburtstag gewidmet.

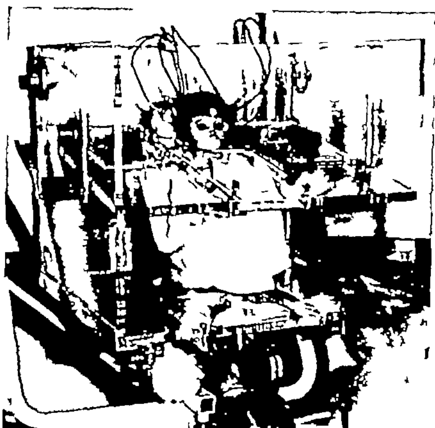


ABB. 1 Typische Positi n de Aff während der Versuche Die Tiere sitz mit ante-flektiertem Kopf in d m K fig au Plexiglas. Bei d n Drehprüfu gen werden die Auge mit H ftpflaster zugekl bt und der Raum verdunk lt

METHODIK

Acht Rhesusaffen (5 ♂ 3 ♀) wurden ohne vorherige Narkotikagaben (*bei Bedarf im Dunkelraum Augen mit Heftpflaster verschlossen*) un tersucht Die Tiere waren während der Versuche sitzend in einem Käfig aus Plexiglas[1] fixiert Durch ein Beißbrett wurde der Kopf um etwa 30 anteflektiert Der Käfig konnte auf einem Drehstuhl montiert werden (Hersteller Dr Ing J F Toennies Freiburg/Br) welcher elektronisch kontrollierte Beschleunigungen ab 0 2 sec² erlaubt Ein zweikanaliger Ny stagmograph (Dr Toennies, Frbg) registrierte die Augenbewegungen, welche über Einsteckelektroden aus Platindraht abgeleitet wurden Die Elektroden waren *für die horizontalen Augenbewegungen bitemporal und* für die vertikalen oberhalb und unterhalb eines Auges (oder auf dem Na senrücken) eingestochen

Wir registrierten Spontannystagmus, optokinetischen Nystagmus das Auftreten von Nystagmus bei der Schwellensuche und postrotatorischen Nystagmus Bei einem Teil der Versuche wurden die horizontalen Augen

[1] Für di Überlassung des Käfigs ind wi H rrn Prof Dr R Jung, Abtlg f Klin. N urophysiol gie d. Univ Freiburg/Br H s str 9 A seh dankba

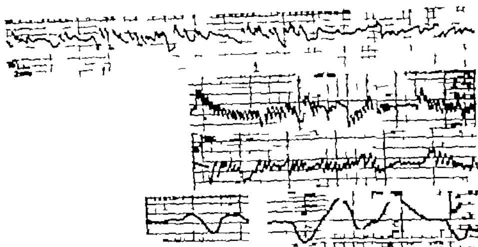


Abb. 2.) Schwellenwerttest beim Affen N 3. Beschleunigung 0,2° sec² Bei ↑ Ende der Beschleunigung, bei ↑ Beginn der Deceleration. b) Horizontaler optokinetischer Nystagmus bei Tier N 9 Reizgeschwindigkeit 60 /sec.) Pendeldeviationen bei Aff K 2 nd N 7

bewegungen simultan durch in der gleichen Ebene befestigte Einsteckelektroden und Klebeelektroden abgeleitet

Eine exakte Eichung war nicht bei jedem Tier möglich nur einige der Tiere folgten den Eichmarken (projizierte Lichtpunkte) gut. Die dabei registrierten Ausschläge waren bei verschiedenen Tieren praktisch gleich groß, weswegen sie für alle zugrundegelegt wurden. Amplitude und Winkelgeschwindigkeit wurden aus mehreren Werten ermittelt, welche frühestens 3, spätestens 10 sec nach dem Stop lagen

ERGEBNISSE

Spontanerscheinungen

Spontannystagmus war nie nachzuweisen Bei langer Versuchsdauer oder starker Ermüdung traten Pendeldeviationen auf (2 Fälle) Sie hatten ähnlich wie beim Menschen eine Frequenz von 0,2–0,3/sec. *Die vestibuläre* Schwelle lag bei diesen Tieren h her Auch Gegenrucke wurden beobachtet, besonders bei Registrierung mit geöffneten Augen

Optokinetischer Nystagmus

Als Reiz diente das bei klinischer Prüfung übliche Streifenmuster das ein vor einer Lichtquelle rollierendes Gitter auf eine halbrunde Projektionswand wirft. Die Streifen wurden mit 30 60 und 90 /sec bewegt. Die meisten Tiere fixierten das Streifenmuster gut und hatten deutlichen Nystagmus. Manche mußte man durch Weckreize aufmerksam halten. Am regelmäßigsten war der ON bei einer Reizgeschwindigkeit on 60 sec.

TABELLE 1

Postrotatorischer Nystagmus

Nr		Schwelle in °/sec	Dauer in sec re	Dauer in sec li	Schlagzahl re	Schlagzahl li	Amplituden in Grad re	Amplituden in Grad li	Winkelgeschw. der langsamen Phasen in °/sec re	Winkelgeschw. der langsamen Phasen in °/sec li
1	Manfred ○	0,3	30	25	48	51	31	31	96	96
2	Fritz ♂	0,5	40	20	12	32	30	43	95	63
3	Gretel ♀	0,2	60	65	90	89	29	27	52	47
6	Gert ♂	0,2	13	45	54	37	47	42	85	0
7	Günther ♂	1,2	30	35	56	55	57	49	118	150
8	Clara ♀	0,2	53	44	66	47	27	23	41	50
9	Ede ♂	0,2	40	28	73	59	42	32	99	103
10	Katharina ♀	0,2	39	42	52	43	33	19	30	37
	Mittelwert	0,37	41	38	56	52	37	33	—	77

Die Winkelgeschwindigkeiten der langsamen Phasen schwankten dabei zwischen 18 °/sec und 85 °/sec, der Mittelwert lag um 50 °/sec ohne bemerkenswerte Seitendifferenzen. Bei Reizgeschwindigkeiten von 30° und 90 °/sec lagen die durchschnittlichen Winkelgeschwindigkeiten beide Male um 40°, erreichten also auch bei bedeutend schnellerer Bewegung des Reizmusters nie die Schnelligkeit der Reaktion auf eine Reizgeschwindigkeit von 60 °/sec. Die Befunde entsprechen in etwa den Verhältnissen beim Menschen. Der vertikale optokinetische Nystagmus war nie besonders deutlich. Nach oben zeigte er größere Regelmäßigkeit als nach unten.

Postrotatorischer Nystagmus

Die Nystagmusreaktion nach Stop aus 90 °/sec (Beschleunigung 0,9 °/sec²) war immer kräftig. Die ermittelten Werte für Dauer, Amplitude, Schlagzahl und Winkelgeschwindigkeit der langsamen Phasen schwankten bei den einzelnen Tieren bis auf wenige Ausnahmen nur im Rahmen einer normalen Schwankungsbreite. Beim Affen Nr. 2 (s. Tabelle) waren Dauer und Schlagzahl des postrotatorischen Nystagmus stark different. Dieses Tier war bei der Ableitung sehr müde (Pendeldeviation, erhöhte Schwelle), was eine Erregbarkeitsschwankung bedingt haben könnte. Gesamtamplitude und Winkelgeschwindigkeit der langsamen Phasen näherten sich im Seitenvergleich aber auch bei diesem Tier dem Normalen.

Der Mittelwert der Nystagmusdauer betrug um 40 sec, der Frequenz um 53 Schläge und der Amplitude um 35°. Die Winkelgeschwindigkeit der langsamen Phasen lag zwischen 30 °/sec und 150 °/sec (Mittelwert 76,5 °/sec). Ein P_{II} trat nur bei einem Tier (Nr. 10) auf und zwar 85 sec nach dem Stop.

Rotatorischer Schwellenwerttest

Die vestibuläre Schwelle lag bei den Affen durchschnittlich unterhalb einer Beschleunigung von 0,37. Leider erlaubte unsere Apparatur keine Beschleunigungen unter 0,2 sec². Bei dieser Andrehgeschwindigkeit hatten 3 von unseren 8 Affen bereits regelmäßigen Nystagmus. Er trat manchmal mit einiger Latenz auf (bis zu 30 sec), was darauf schließen läßt, daß diese Beschleunigung schwellennah war. Die beiden Affen mit der wesentlich erhöhten Schwelle (Nr. 2 und Nr. 7) waren offensichtlich ermüdet. Sie zeigten Pendeldeviationen, welche auch bei ermüdeten Menschen vermehrt auftreten. Wenn man diese Tiere weckte, oder sie von sich aus unruhig wurden, trat für kurze Zeit schon bei geringeren Beschleunigungen Nystagmus auf.

Berücksichtigt man bei der Berechnung des Mittelwertes die beiden genannten Tiere (Nr. 2 und Nr. 7) nicht, so liegt die vestibuläre Schwelle für Rhesusaffen um oder unter 0,2 sec². Dieser niedrige Wert war nur durch die Einsteckelektroden zu erreichen. Bei gleichzeitiger Ableitung mit Klebeelektroden trat erkennbarer Nystagmus erst bei um etwa 0,2 sec² höheren Beschleunigungen auf.

BESPRECHUNG

Rhesusaffen eignen sich gut zu nystagmographischen Untersuchungen. Ihre vestibulären und oculomotorischen Reaktionen sind mit denen des Menschen nah verwandt. Die im Käfig fixierten Tiere verhalten sich meistens ruhig. Man muß nur gegen ihre starke Ermüdbarkeit angehen, die bei längerer Versuchsdauer die Ergebnisse verfälschen kann. Auf der anderen Seite ist bei gut reagierenden Tieren sogar eine exakte Eichung der Blickbewegungen möglich.

Einsteckelektroden haben sich sehr bewährt. Das Kurvenbild wird sehr ruhig, weil kaum Artefakte auftreten. Durch die Umgehung des Hautwiderstandes können schon kleine Potentialschwankungen erfaßt werden. Die Nystagmogramme werden bei Rhesusaffen auch deshalb empfindlicher, weil die Bulbi wegen der dünnen temporalen Knochenschicht sehr dicht an der Stelle liegen, wo das *Potential abgegriffen wird.*

SUMMARY

The vestibular threshold in 6 conscious monkeys was reached during an acceleration 0.2 °/sec². (Two tired animals had been eliminated.) Duration (40), frequency (63 beats), amplitude (35) and deflection of the slow nystagmus-phase (76.5 /sec) were counted. Eye movements were registered electronystagmographically using platinum-electrodes placed under the skin.

LITERATUR

TER BRAAK, J W 1937 Über di Empfindlichkeit des Boge gangsapparates für Winkel beschleunigungen *Pflueger Arch Ges Physiol 238* 319

BUYS, E., und RIJLANT P 1930: Le seuil d excitation (accélération angulaire) des canaux semi-circulaires. *Arch Int Physiol 49* 101

DECHER, H 1962: Zur Bestimmung der Drehreizschwell bei Norm lfällen. *Z Laryng Rhinol Otol 41* 838

EX, J JONGKEES, L. B W und KLIJN, J 1950 Th threshold of the vestibular organ. *Acta Ot laryng* (Stockh) *50* 292.

JONES, G M BOST W und KOWALSKY N 1964 Dynamics of the semi-circular canal compared in yaw pitch and roll *Aerospace Med 35* 984

KORNHUBER, H H 1967: Physi logi und Klinik des zentralvestibulären Systems (Blick und Stüt motorik) In *Handbu h d r HNO-Heilkunde* Bere des-Link Zöllner (Her au geber) III/3 Georg Thieme Verlag Stuttgart 1966

MITTERMAIER, R und ROSSBERG, G 1936: Vestibularisuntersuchungen mit schwellennahen Beschl unigungsreiz n *Arch Ohr Nas K hlk pfheilk 168* 313

MONTANDON A., und RUSSBACH, A 1955 L épreuv girat ire liminaire *Pract Otorhinolaryng* (Basel) *17* 224

MOWRER, O H 1935: zit n te Braak *J Comp Psychol 19* 177

PFALTZ, C. R 1960: Diskussi n bemerku g *C nfin N urol 20* 259

RUSSBACH, A 1955 Le seuil du nystagmus d'accélération giratoire *Thèse* Genève

Dr G Lang Univ Klinik fü HNO-Krank
Freiburg i Br Killianstraße Deutschland

Eingegangen am 8 De mber 1966

Acta oto-laryngologica 64 [illegible] (1967)

LATENCY TIME OF VESTIBULAR NYSTAGMUS IN REPEATED BIDIRECTIONAL ROTATORY STIMULATION

E. Fluur, L. Mendel and L. Lagerström
Stockholm, Sweden

From the Department of Otolaryngology (Head: Prof. C. A. Hamberger) Karolinska Sjukhuset, Stockholm

A total of 77 healthy subjects underwent repeated, identical acceleration in the clockwise and counterclockwise directions. The case material was divided into three groups, with stimulation at 1°/sec², 3°/sec² and 5°/sec². The latency times are found to increase somewhat in successive stimulation in alternating directions at 1°/sec², whereas no such increase is noted at 3°/sec² and 5°/sec². The coefficients of variation of the latency times are similar in all groups. The mean differences between the latency times in clockwise and counterclockwise acceleration in the different groups is almost constant in repeated tests.

It is concluded that one rotatory test does not affect the result of the next test. It is also found that the latency times are somewhat longer in clockwise acceleration than in counterclockwise, irrespective of the direction of the first acceleration. Altogether 33% of the subjects have shorter latency time in acceleration in one direction in all three tests. In 9% this applies to clockwise acceleration and in 24% to counterclockwise acceleration.

The peripheral vestibular apparatuses are in connexion with each other through several conducting pathways, which implies that they have a possibility of exerting a mutual influence in various ways, both direct and indirect. Sala (1965) demonstrated, in experimental animals, that one labyrinth can inhibit the activity of the other via the efferent pathways. In previous studies in man, we found that repeated monolabyrinthine stimulation—caloric or rotatory—can influence the mode of reaction of the contralateral labyrinth in essentially two ways, i.e., either in a facilitating or an inhibitory direction (Fluur & Mendel 1962, 1963).

It has also been discussed in the literature whether on stimulation of both labyrinths, one obtains the same response bilaterally or whether there is a preponderance of one side. Thus, Jongkees (1948) found, in a cupulometric study of about 100 healthy subjects, that 3% had a preponderance to the right side and 13% to the left. Other authors have considered that

such vestibular imbalance might depend on an initially-elicited nystagmus in one direction producing a greatly increased nystagmus in the opposite direction in the next test due to a secondary nystagmus phase deriving from the initial test (Aschan *et al.*, 1952 Mittermaier & Rossberg 1956 Stahle 1957)

In recent years individual rotatory tests have often disclosed a preponderance to the left, in agreement with Jongkees finding at cupulometry (Pfaltz, 1960 Decher 1964) The results obtained in so-called threshold tests have on the contrary shown that no difference is present between nystagmus directed to the right and to the left (Decher 1962 1964 Ham, 1964)

Opinions have thus been at variance and no definite answer to the question has been forthcoming We have studied the duration of the latency time of nystagmus in per rotatory stimulation of varying strength (Fluur & Mendel 1966) In addition we have investigated the extent to which repeated stimulation in one direction results in reproducible latency times (Fluur Mendel & Lagerström 1966) No signs of fatigue or habituation were demonstrable in these investigations

The object of the present investigation was

1 To ascertain whether rotatory stimulation in one direction influences the latency time of vestibular nystagmus elicited by rotation in the opposite direction

2 To study whether in weak rotatory stimulation any directional preponderance is demonstrable on per rotatory recording of the latency time of nystagmus

CASE MATERIAL

The studies were made in three groups of 20 experimental subjects in each and one group of 17 subjects All were right handed and their age ranged from 20–40 years All were clinically healthy both as regards their history and from the otoneurological point of view In every respect the composition of the material was the same as in our previous studies.

METHOD

We used a rotation chair with equipment for centric fixation of the subject's head The chair could be rotated at given rates, and could be accelerated and decelerated with exactly adjustable strengths of stimulation

Mode of Stimulation

During the tests the experimental subject sat with his head flexed 30 forwards, and fixed in a special holder When the performance of the chair had been calibrated and the recording arrangement checked the chair was

accelerated clockwise at 1 /sec² until distinct nystagmus was observed After 2 min at a constant speed, the chair was decelerated at 1 /sec² until it had reached a speed of about 10 /sec² The object of this acceleration and deceleration was to acquaint the subject with the procedure and also to make a final check of the apparatus.

The actual test was then started, using different speeds of acceleration until nystagmus was observed, after which the speed was kept constant for 2 min. This was followed by deceleration until nystagmus was once again elicited this was succeeded by rotation at a constant speed for at least 2 min One acceleration and one deceleration comprised one test, and the whole examination consisted of three tests The chair was never ac celerated from a standstill but always from about 10 /sec² since this ensured an absolutely smooth start of acceleration

The case material was divided into three stimulation groups, i.e., initially rotated clockwise at 1 3 and 5 /sec² and a fourth group initially rotated counterclockwise at 3 sec²

RESULTS

A study of Table 1 shows that the latency times were longest in group 1 and then decreased successi ely in groups 2 and 3 Accordingly the mean values are successively lower

The hanges in the latency times in each group in the three consecutive tests can be followed in Tables 1 and 2 It i then seen that mean latency f the three stimulation rates varied merely by about one sec. An inappreci able successi e increase occurred only in counterclockwise acceleration at 1 nd 5 /sec² No corresponding succes ive decrease can be discerned

The calculated coefficient f variation of the groups latency times in each direction i gi en in the table. In group 1 the values are 0 66 and 0 68 for clockwise and counterclockwise acceleration, respectively The corresponding lues in the ther groups are 0 67 and 0.53 0 68 and 0 62, 0.5 a d 0.53. Thus, no great difference is present between the various groups Within the groups, a successi e increase can be n ted in group 1 which cann t be observed in the other groups.

Purely individually th variations in th different measurements are somewhat hard to judge Certain subject consistently had the same latency times in all three tests, whereas others varied greatly No regularity what soever could be demonstrated in this respect.

If we now consider the relation between the latency time in clockwise and in counterclockwise acceleration, it is seen from the mean values in all groups that the time was shorter in the latter This does not, however apply to the second test in group 1 The mean difference between the lat ncy times in clockwise and counterclockwise acceleration is 5.5 5 45 and 6.1 sec in the three tests in group 1 2.25 3.07 and 2.25 sec in group 2,

9 Gram

TABLE 1 *Latency time (sec) on stimulation at 1 3 and 5 /sec² clockwise acceleration before counterclockwise in all tests*

Cw clockwise, CCw counterclockwise

Test	1			2			3			Arithmetic mean in 3 tests	
Direction of acceleration	Cw	Diff Cw + /CCw −	CCw	Cw	Diff Cw + /CCw −	CCw	Cw	Diff CW + /CCw −	CCw	Cw	CCw
Group I											
Acc. 1 /sec²	3	− 3	6	3	−12	15	3	−10	15		
	20	+16	4	7	+ 4	3	15	+12	3		
	7	+ 2	5	3	− 7	10	1	− 3	1		
	6	0	6	11	−10	21	11	− 3	11		
	3	− 8	11	3	0	3	1	− 7	11		
	5	− 5	10	3	−10	13	2	− 8	10		
	10	− 2	12	7	0	7	1	− 8	12		
	16	+ 8	8	6	0	6	11	+12	2		
	13	+ 8	5	15	+ 6	9	7	+ 2	5		
	18	+15	3	20	+ 7	13	22	+ 8	11		
	0	+ 1	5	13	+10	3	15	+11	1		
	24	+10	14	11	+ 5	6	18	+15	3		
	8	− 4	12	12	+ 0	3	3	− 3	6		
	10	+ 2	8	12	− 0	18	13	+ 1	12		
	1	1	2	2	− 3	5	0.5	− 1.5	2		
	9	+ 1	5	0	+ 4	2	7	− 1	8		
	12	+ 8	4	2	− 5	7	5	+ 1	1		
	7	− 0	13	9	+ 7	2		− 1	0		
	10	− 6	15	5	− 3	8	22	− 8	30		
	12	+ 1	11	1	− 1	5	8	− 3.5	11.5		
Mean latency	10.2		8.0	7 7		8.1	9 1		8 7	9 0	8.2
Coeff of variation of latencies	0 58		0 48	0 6[illegible]		0 73	0 80		0 81	0 66	0 68
Mean diff. latency Cw/CCw		5 6			5 16			6.1			
Coeff of variation of diff latency Cw/CCw		0 70			0 66			0 71			

Group 8 Acc. 3°/sec²										Arithmetic mean 1–3 test	
										Cw	CCw
	3	0	3	2	0	2	2	− 2	4		
	4	0	4	5	2	7	4	+ 1	3		
	1	2	3	3	0	2	4	+ 1	3		
	9	+ 6	3	8	+ 3	5	15	+ 10	5		
	2	0	3	1	− 3	7	5	− 1	6		
	2	0	2	1	2	3	1	− 3	4		
	10	± 5	5	11	+ 7	1	3	− 3	6		
	3	1	4	7	+ 6	1	5	+ 1	1		
	1	4	8	1	2	3	3	− 2	5		
	4	+ 1	2	3	± 2.5	0.5	1	− 3	1		
	4	+ 1	3	1	− 7	8	1	− 2	3		
	6	+ 1	5	1	2	2	1	− 1	2		
	2	− 5	7	2	+ 1	1	2	+ 1	1		
	3	+ 1	2	6	+ 2	1	4	0	4		
	5	+ 1	4	5	+ 2	3	3	+ 2	1		
	9	+ 7	2	6	+ 2	4	7	+ 1	8		
	5	+ 1	4	0	+ 1	5	0	+ 3	6		
	10	+ 7	3	9	+ 1	5	1	+ 2	2		
	8	− 2	11	15	+ 10	5	8	+ 2	6		
	1	1	2	7	0	7	6	+ 1	2		
Mean latency	4.7		1.0	5.4		3.9	4.4		3.9	1.9	3.9
Coeff. of variation of latencies	0.63		0.58	0.89		0.36	0.77		0.43	0.67	0.83
Mean diff. latency Cw/CCw		2.35			3.07			2.25			
Coeff. of variation of diff. latency Cw/CCw		1.00			0.85				0.90		

Cw clockwise; CCw counterclockwise

Test	1			2			3			Arithmetic mean in 3 tests	
Direction of acceleration	Cw	Diff Cw + /CCw –	CCw	Cw	Diff Cw + /CCw –	CCw	Cw	Diff CW + /CCw –	CCw	Cw	CC
Group 3											
Acc. 5 /sec²	7	+ 5	2	1	+ 3	1	10	+ 7	3		
	5	+ 3	2	5	+ 2	3	4	+ 2	2		
	3	0	3	5	+ 2	3	1	+ 1	3		
	3	0	3	3	+ 1	2	3	+ 1	2		
	3	+ 1	2	1	+ 3	1	3	+ 2	1		
	2	0	2	0	+ 1	5	1	– 1	2		
	2	+ 1	1	1	– 1	2	2	0	2		
	2	– 1	3	1	+ 3	1	2	– 1	3		
	4	+ 2	2	2	– 2	1	0	+ 2	1		
	2	0	2	5	– 1	9	7	– 3	10		
	2	+ 1	1	3	+ 2	1	10	+ 1	6		
	6	0	6	1	– 1	2	1	– 2	3		
	4	+ 2	2	2	0	2	1	– 2	3		
	3	– 1	4	2	– 1	3	1	– 1	2		
	1	– 1	2	1	– 1	2	1	– 1	2		
	2.5	– 1.5	4	4	+ 2	2	2	– 1.5	3.5		
	3.5	+ 1.5	2	1	– 3	1	5	+ 3.5	2		
	11	+ 9	2	1	– 1	2	1.5	– 0.5	5		
		+ 3	2	5	+ 2	3	2.5	+ 1.5	1		
	9	+ 7	2	6	+ 1.5	1.5	11	+ 0	1.5		
Mean latency	4.0		2.5	3.3		2.7	1.0		3.1	3.8	2.7
Coeff of variation of latencies	0.64		0.40	0.55		0.69	0.81		0.66	0.68	0.62
Mean diff. latency Cw/CCw		2.0			1.08			2.33			
Coeff of variation of diff latency Cw/CCw		1.21			0.58			0.90			

TABLE 2 *Latency time (sec) on stimul tion 1 3 /sec² count rclockwise acceleration bef re clockwise in all tests*

Cw clockwise CCw counterclockwise

Test — Direction of acceleration	1 Cw	1 Diff. Cw/CCw	1 CCw	2 Cw	2 Diff. Cw/CCw	2 CCw	3 Cw	3 Diff. Cw/CCw	3 CCw	Arithmetic mean in 3 tests Cw	Arithmetic mean in 3 tests CCw
Group 4 Acc 3°/sec²	5	1.5	6.5	1	1.5	2.5	7	1	6.0		
	2	1	6	10	5	3	3	+1.5	1.5		
	6	1.5	1.5	8	1.5	3.5	1.5	−2.5	1		
	3	0.5	3.5	3	0	3	2	−1	3		
	2.5	0	2.5	2.5	1	1.5	3	+1	2		
	1.5	0	1.5	2.5	1	1.5	1.5	2	3.5		
	3	1	4	3	0.5	2.5	1	1	3		
	6	0.5	5.5	3.5	1	1.5	2.5	+0.5	2		
	1	0	4	5	1	0	4	+1.5	3.5		
	5.5	1	1.5	7	3	4	3	0.5	3.5		
	8	2.5	4.5	4.5	+1.5	3	4	+3	1		
	7.5	4.5	2	5.5	2.5	3	3.5	−1	7.5		
	4	0	4	6	1.5	2.5	7	+4.5	2.5		
	2	3.5	6.5	6	+2	1	11	+2	9		
	6	0	6	1	+2.5	1.5	11	2.5	8.5		
	8	0	8	1.5	0	1.5	5	+2	3		
	11	3.5	7.5	1	+2	2	6	0	6		
Mean latency	3.1		4.5	6.0		3.0	4.7		1.0	1.9	3.8
Coeff. of variation of latencies	0.50		0.45	0.40		0.12	0.62		0.62	0.57	0.53
Mean diff. latency Cw/CCw		1.8			2.1			1.8			
Coeff. of variation of diff. latency Cw/CCw		1.01			0.93			0.68			

and 2.0 1.98 and 2.33 sec in group 3. In group 4 the difference is of the same order of magnitude as in group 3 (1.8 2.1 and 1.8 sec). The coef*ficients of variation of the mean differences, listed in Table 1 show the variations between the different individuals.* These are higher at the higher intensities of stimulation.

Different individuals showed a highly variable pattern in the three *consecutive tests. We investigated in each subject whether any* consistent tendency to a shorter latency time existed in one direction of acceleration in all three tests. We then found that 4 subjects in group 1 had a shorter *latency time in clockwise* acceleration and 5 in counterclockwise whereas the remaining 11 in the group varied indecisively. In group 2, one subject had a shorter latency time in clockwise acceleration 5 in counterclockwise *and 14 were indecisive. In group 3 the corresponding figures were* 2, 6 and 12 subjects. In group 4 none of the subjects had a consistently shorter latency time in clockwise acceleration whereas this applied to 3 subjects *in the* counterclockwise direction.

Thus, in the whole material we found that of 77 subjects a total of 26 consistently had a shorter latency time in all three tests with acceleration in one direction. In 7 subjects (9%) this applied to clockwise rotation and in 19 (24%) to counterclockwise rotation. In this respect group 4 did not differ from the other groups.

DISCUSSION

One of the objects of the present study was to ascertain whether rotatory stimulation in one direction influences the latency time of nystagmus elicited *by rotation in the opposite direction. The results will therefore* be compared with those obtained in repeated stimulation in one direction only (Fluur & Mendel 1966). In the present study we found that the *latency times in the initial accelerations at 1 sec² varied around a mean* of 10.2 sec in clockwise rotation and around 8.0 sec in counterclockwise rotation. The corresponding means at 3 /sec² and 5 sec² were 4.7 and *4.0 sec and 4.0 and 2.5 sec respectively. In our earlier studies* with clockwise rotation only the corresponding figures were 8.6 6.1 and 2.2 sec. Despite the fact that in these cases the tests were made in the same way partly deviating results were unquestionably obtained particularly in group 2. We interpret this as a further example of great individual differences in the excitability of the vestibulo-ocular reflex are a matter that we have already pointed out in previous papers.

The investigation has clearly shown that the mean latency times vary in such a way that no successive inhibition or facilitation seems to exist. It seems evident that comparatively strong rotatory stimuli are required to elicit habituation since it was not produced by any of the per rotatory stimuli used. Even the considerably stronger stimulation in the form of

braking to a standstill from a constant speed of 30 /sec repeated 10 times, failed to elicit any calorically demonstrable habituation (Fluur & Mendel, 1963) This affords additional support to our present observations.

Although large variations are occasionally reflected in the individual readings, they do not indicate that one test affects the results of the next. Groups 2 3 and 4 do not deviate from the given pattern, and show good stability in repeated measurements. The results in group 1 are somewhat puzzling, in that the means of the latency times show uniform values, wherea their coefficients of variation increase successively In earlier studies, with acceleration in one direction, a tendency to decreased coefficients of ariation were found in certain cases. The changes were not, how ever of such an order of magnitude that they were significant Possibly a calculation of the standard deviation (S.D) in each subject would have clarified the matter but since only three measurements were made, we considered that the results would be too uncertain to permit any reliable conclusions.

The differences between the latency times in clockwise and counterclock wise cceleration decrease with rising strength of stimulus, in the same way as the latency times. This merely reflects what could be expected. The somewhat larger coefficients of variation in groups 2, 3 and 4 are more surprising Since these coefficients are a measure of the relative variability there is a comparatively large S.D of the differences between the latency times in clockwise and in counterclockwise rotation. Although the measurement to within 1 sec is a factor to be taken into account in this connexi n, it does not suffice as an explanation.

The coefficients of variation of the differences reflect the variations in th individual latency times. Thi also applies to the differences between the latency times measured which, in some individuals, are large in some test and maller in others The large difference between the coefficients of variation in the three tests in group 2 is dependent, to a great extent, on the fact that, in some tests, a few ubjects did not respond by nystagmus until after a trikingly long latency We ha e often noted such long latencies, nd have interpreted them as due to purely normal but extremely strong influ nces on the vestibulo-ocular reflex path, elicited centrally Frequently only one test is affected, and values adequate for the strength f t mulu are obtained in the subsequent ones.

When analyzing the material, we also in estigated whether the latency t mes were consistently shorter or longe on acceleration in one direction than in the opposite direction If this were to be the case it would reflect in sensit ve w y a constant estibulo-ocular imbalance, i.e preponder ance On weak acceleration at 1 /sec² we found no difference between the l t ncy times in lockwise and counterclockwise acceleration, wherea such a difference was present in the other groups.

Since both the second and third test were included in these calcula tions, and group 4 was also included a a control group, the results must

be regarded as unquestionable Unquestionably such preponderance is present in comparatively few subjects and it is understandable that the data in the literature vary since less sensitive *methods of examination* have been used (Milojevic & Watson 1965) Moreover studies with per rotatory stimulation have not previously been focused directly on these problems (Decher 1962 1964)

The results in the present study consistently showed that the latency times varied groupwise with the coefficients of variation these being around 0.50–0 70 They are of the same order of magnitude as in our earlier studies, using repeated unidirectional stimulation Stimulation in *alternating directions of acceleration* evidently *results in the same latency* times as in repeated stimulation in one direction This implies that we have a uniform s D factor in our per rotatory studies Moreover the latency times lie on approximately the same level as in our earlier studies

In our normal material the distribution at each given strength of stimulus is such that the longest latency times noted were 30 sec at 1 /sec² 15 sec at 3 /sec² and 11 sec at 5 /sec² These maximum values represent approximately three times the arithmetic mean Consequently this affords a possibility of fixing a clinical borderline value for the latency times regarded as normal, the remaineer being regarded as suspectedly pathological This applies especially if the latency times exceed these fictitious borderline values in repeated tests

ZUSAMMENFASSUNG

77 gesunde Menschen sind mit wiederholten identischen Beschleunigungen nach rechts und links untersucht worden Wir benutzten gruppenweise die Beschleunigungen 1 /sec² 3 /sec² und 5 /sec und fanden dass die Latenzzeit bei sukzessiven richtungsalternierenden Stimulationen mit 1 /sec² zunahm, wohingegen eine solche Zunahme bei 3 /sec² und 5 /sec² nicht zu finden war Der Variationskoeffizient der Latenzzeiten der Gruppen verhielt sich gleichartig. Die mittlere Differenz zwischen den Latenzzeiten bei Rechts und Linksbeschleunigung ist gruppenweise beinahe konstant bei wiederholten Untersuchungen

Der Schlussatz der Untersuchungen ist dass ein rotatorischer Test den nachfolgenden nicht beeinflusst Wir haben auch gefunden dass die Latenzzeiten bei Rechtsbeschleunigung etwas länger sind als bei Linksbeschleunigung unabhängig von Richtung der ersten Beschleunigung 33% hatten bei sämtlichen 3 Testen kürzere Latenzzeit bei Beschleunigung in einer Richtung 9% bei Rechtsbeschleunigung und 24% bei Linksbeschleunigung

REFERENCES

ASCHAN G NYLÉN C. O STAHLE, J and WERSÄLL, R 1952 The rot ti n test *A ta Ot larng* (Stockh) *1* 451

D CH R, H 1962: Zur Bestimmung d Drehreizschw ll n bei N rm lfall n *Z Laryng Rhinol Otol* *41* 838.

— 1964 Zu Technik nystagmographischer Untersuchungen Z Laryng Rhinol Otol 43, 757
— 1964 Z m Richtungsuberwiegen des xperimentellen Nystagmus bei Normalfällen. Arch Ohr Nas. K hlk pfheilk., 183 285.
Fl u, E., nd Mendel, L., 1962: Habituati n, efference nd estibular interpl y I Monaural caloric habituation. Acta Otolaryng (Stockh.) 55 65.
— 1962 Habituation, efference nd estibular interplay II Combined caloric habit tion. Act Otolaryng (Stockh.) 55, 136.
— 1963 Habituation, efference and vestibular interplay III U idirectional rotatory habituation. Act Otolaryng (Stockh.) 57 81
— 1966 Rel tion between strength of stimulus nd duration of l tency time i estibular rot tory ystagmus. Act Otolaryng (Stockh.) 61 163.
Fl u, E., Mendel, L., nd Lagerström, L., 1966 Reproducibility f duration of l tency time m nidirect onal per-rotatory ystagmus. Act Otolaryng (Stockh) 62 118
Haas, E., 1964 Zur Frage der Altersabhängigkeit der Drehreizschwelle. Z Laryng Rh nol Otol., 43 228
Jovanders, L. B. W 1948 Value of the caloric test f the labyrinth. Arch. Otolaryng (Chic.) 48 402.
Milojevic, B., nd W tson, J L., 1965 Vestibular symmetries i right nd left handed people Act Otolaryng (Stockh.) 60 322.
Mittermaier, R nd Rossberg, G., 1956 Vestibularisuntersuchungen mit schwellennahen Beschleu igungsreizen. Z. H ls Nas. Oh K hlk pfheilk., 168 313.
Pfaltz, C. R., 1960 Die nystagmographische Erfassung est bulärer Labyrinthreaktionen. Bull Schweiz. Akad Wiss., 16 221
Sala, O., 1965 The efferent estibular system. Act Otol ryng (Stockh.) Suppl. 197
S ahle, J 1957 Electro ystagmography i the rotatory test. Upsal läkar före ings fö handl., 17 77

Dept f Otolaryngology
Karol nsk Sj khuset,
Stockholm 60 Sweden

Received Decembe 6 1966

Acta oto-laryngologica 64 136–145 (1967)

ÜBER DEN EINFLUSS DER VISKOSITÄT IN DEN SCHNECKENKANÄLEN

K. Schöberl
Wien, Österreich

Der Einfluß der Viskosität auf die Wellen in den flüssigkeitsgefüllten Kanälen der Schnecke (von den perilymphatischen Skalen bis zu den Tunnelräumen) wird in Abhängigkeit von einer einzigen Kennzahl (Dicke der akustischen Grenzschicht/hydraulischer Radius) dargestellt. Dieser Einfluß nimmt mit abnehmender Frequenz und mit abnehmenden Querschnittsabmessungen zu. Es verringert sich dadurch die Längsverschieblichkeit der Flüssigkeit und damit die Querbewegung der Kanalwände, ferner entsteht eine Längsscherkraft und damit eine Tendenz zu Längsbewegungen der Kanalwand. Die Wellendämpfung infolge der Viskosität und die Folgen einer Änderung der Größe der Viskosität gegenüber den normalen Meßwerten werden diskutiert.

1. *Einleitung*

Die Frage, wie sich die Viskosität der Innenohrflüssigkeiten auf die Schwingungsvorgänge in der Schnecke auswirkt, ist bereits mehrfach gestreift worden (Jung 1940, Fletcher 1951, Ranke 1953); im folgenden soll diese Frage ausführlicher, auch unter Berücksichtigung der Vorgänge in den engeren Kanälen (z. B. im Canalis spiralis) untersucht werden. Der Viskositätseinfluß wird dabei in Abhängigkeit von der Kennzahl δ/R_h dargestellt. δ ist die Dicke der akustischen Grenzschicht (im Abschnitt 4 näher erläutert), die sich aus $\delta = \sqrt{\nu/\pi f}$ (ν kinematische Viskosität in cm² sec⁻¹, f Frequenz in Hz) bestimmt. R_h ist der hydraulische Radius des Kanalquerschnittes, wobei $R_h = 2S/U$ ist (S Fläche, U Umfang des Kanalquerschnittes).

2. *Viskosität der Innenohrflüssigkeiten*

Im Gegensatz zu früheren Auffassungen (Ranke 1953, S. 107) geht aus neuen Messungen (Rauch 1964, S. 108) hervor, daß die Viskositäten der Peri- und der Endolymphe ungefähr gleich groß sind und das 1,02- bis 1,03fache der Viskosität des Wassers betragen. Über die Viskosität der zwei weiteren Innenohrflüssigkeiten (subtectoriale Lymphe und Cortilymphe, Rauch 1964, S. 302) ist derzeit nichts bekannt.

Im folgenden wird die Viskosität sämtlicher Innenohrflüssigkeiten gleich der Viskosität des Wassers bei 37° C (= 0,7080 cP, siehe d'Ans u. Lax 1949, S. 1094) angenommen (vgl. jedoch Abschnitt 7). Mit $\varrho = 1{,}02$ g cm⁻³ (spezifische Masse der Peri- und Endolymphe nach Rauch 1964, S. 454) und ν = (Viskosität in cP/100ϱ) cm² sec⁻¹ wird dann $\nu = 0{,}0077$ cm² sec⁻¹, womit Tabelle 1 berechnet wurde.

TABELLE 1 *Dicke δ der akustischen Grenzschicht in μ* (= 10 cm)

f	12,5	25	50	200	800	3 200	12 800 Hz
δ	141	99	70	35	17,5	8,8	4,4 μ

3 *Abmessungen im Schneckenquerschnitt*

Tabelle 2 bringt die für den Viskositätseinfluß wichtigen Abmessungen für jeweils einen basisnahen und einen spitzennahen Querschnitt, der den Orten der maximalen Trennwandauslenkung bei $f = 12\,800$ Hz bzw. bei $f = 200$ Hz entspricht (vgl. die Spalte Hz"). R_k bedeutet den Radius des Kreises, der der Querschnittsfläche S des jeweiligen Kanales flächengleich ist ($\pi R^2 = S$); der hydraulische Radius R ist wegen der Abweichung der Querschnittsform von der Kreisform kleiner als R_k. Größe und Form der Kanalquerschnitte sind aus den Abbildungen in Kraus (1933 S 5) und Skudrzyk (1934 S 44) sowie aus Ranke (1953 S 64) und Neubert (1950) abgeleitet.

Aus den Tabellen 1 und 2 kann das Nomogramm in Abb 1 ermittelt werden, das für jede Frequenz und für jeden Schneckenkanal die Kennzahl δ/R angibt.

4 *Längsströmungen*

Man denke sich einen Kanal mit idealer (reibungsfreier) Flüssigkeit (Index i) gefüllt, für die $= 0$ und daher $\delta = 0$ ist; bei Erregung mit einem Sinuston entsteht an einer bestimmten Stelle $x =$ konst. ($x =$ Längskoordinate des Kanals) eine sinusförmig wechselnde Längsströmung. Die — im allgemeinen von Punkt zu Punkt des Querschnittes verschiedene — Geschwindigkeitsamplitude dieser Strömung sei u. Ihr über den Querschnitt gemittelter Wert $\bar{u}$ (Querstriche bedeuten Mittelwertsbildung)[1]. Ersetzt man nun in diesem Kanal die ideale Flüssigkeit durch eine viskose Flüssigkeit (Index v) und nimmt an, daß an der vorerwähnten Stelle $x =$ konst. das gleiche be-

TABELLE 2 *Abmessungen im Schneckenquerschnitt*

Kanal	Hz	R_k [μ]	R_h [ ]	δ/R_h
Perilymphatische Skalen	12 800	737	693	0,0063
	200	439	412	0,085
Ductus endolymphaticus	12 800	320	255	0,0172
	200	320	208	0,168
Canalis spiralis	12 800	40,0	37,6	0,118
	200	69,0	65,0	0,54
Innerer Tunnelraum	12 800	13,8	12,0	0,363
	200	24,0	21,0	1,66
Nuelscher Raum	12 800	11,3	8,0	0,55
	200	19,3	13,8	2,02
Äußerer Tunnelraum	12 800	8,2	4,6	0,92
	200	11,3	8,7	4,08

[1] Die Momentanwerte sind dabei als $2\pi/f$ bzw. als $2\pi/L$.

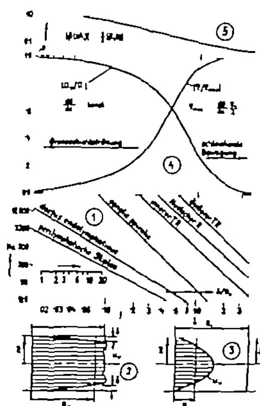


Abb 1 Nomogramm zur Ermittlung der Kennzahl δ/R_k für die Schneckenkanäle (für $\nu = 0{,}0071$ cm²/sec) An der Skala links unten ist die Strecke abzulesen, um die bei n facher Viskosität die Geraden des Nomogramms in Richtung der Abszissenachse nach rechts zu verschieben sind (vgl. Abschnitt 8).
Abb 2. Strömungsverhältnisse bei $\delta/R_k = 0{,}15$ (Grenzschichtströmung)
Abb 3 Strömungsverhältnisse bei $\delta/R_k = 1{,}0$ (schleichende Bewegung) (zu Abschnitt 4).
Abb 4 Verlauf von $|u_w/u_i|$ und $|\;/\tau_{max}|$ in Abhängigkeit von δ/R_k (Abschnitte 3 bis 6).
Abb. 5 Verlauf des Faktors im Abhängigkeit von δ/R_k (zu Abschnitt 8).

schleunigende Druckgefälle $-dp/dx$ herrscht wie bei idealer Flüssigkeit (vgl den Anhang) dann ergeben sich die Geschwindigkeitsamplituden u bzw $\bar{u}_r$

Die Abb 2 und 3 bringen Beispiele hiefür Abb 2 gilt für den Fall $\delta/R_k = 0{,}15$ gibt also wie Abb 1 lehrt die Verhältnisse in den perilymphatischen Skalen bei etwa 80 Hz, im Ductus endolymphaticus bei etwa 250 Hz und im Canalis spiralis bei etwa 6400 Hz wieder Abb 3 (für $\delta/R_k = 1{,}0$) veranschaulicht die Zustände im Canalis spiralis bei etwa 30 Hz im inneren Tunnelraum bei etwa 800 Hz im Nuelschen Raum bei etwa 3000 Hz und im äußeren Tunnelraum bei etwa 10 000 Hz In Abb 2 und 3 ist ein kreisförmiger Kanalquerschnitt ($R_k = R_r = R$) und $2\pi R/\lambda < 1$ (λ = Wellenlänge) angenommen so daß $u_i = \bar{u}$ ist (angenähert ebene Welle) Die Strömungen sind für den Augenblick dargestellt, in dem sie ihren Größtwert erreichen nach einer Halbperiode würden sie in der entgegengesetzten Richtung nach links verlaufen

Abb 2 ist ein Beispiel für die Grenzschichtströmung (vgl den Anhang) bei der durch die Viskosität der Mittelwert der Längsgeschwindigkeitsamplitude nur wenig verringert wird (von $\bar{u}$ auf $\bar{u}$) Der Einfluß der Viskosität

beschränkt sich hierbei wie der Verlauf von u zeigt, im wesentlichen auf die der Kanalwand anliegende akustische Grenzschicht von der Dicke δ während in dem an die Grenzschicht anschließenden inneren Bereich fast dieselben Verhältnisse wie bei idealer Flüssigkeit herrschen. Ganz im Gegensatz hierzu ist bei Abb. 3 dem Fall der näherungsweise schleichenden Bewegung (vgl. den Anhang), dieser innere Bereich infolge des Anwachsens von δ völlig verschwunden. $\bar u$ ist erheblich kleiner als $\bar u$ (In diesem Fall hat δ allerdings keine anschauliche Bedeutung mehr.)

Mit zunehmendem δ/R_h setzt die Flüssigkeit einer Längsverschiebung einen immer größer werdenden Widerstand entgegen, so daß $\bar u$ gegenüber $\bar u$ immer kleiner wird. Unter Voraussetzung eines gleichbleibenden beschleunigenden Druckgefalles ist das Verhältnis $|\bar u_v/\bar u|$ mit den Formeln des Anhanges in Abb. 4 in Abhängigkeit von δ/R dargestellt.

5 *Querbewegungen der Kanalwände*

Gemäß der Kontinuitätsgleichung für einen Kanalquerschnitt S

$$-S\frac{d\bar u}{dx} = U\tilde w$$

ist die über den Kanalumfang U gemittelte Amplitude der Quergeschwindigkeit w der Kanalwand proportional $d\bar u/dx$ wofür, wenn man näherungsweise eine permanente Welle annimmt, $-i(2\pi/\lambda)\bar u$ gesetzt werden kann. Sohin ist mit $R = 2S/U$ $|\tilde w| = \frac{1}{2}(2\pi R_v/\lambda)|\bar u|$ welche Gleichung, einmal für die viskose und einmal für die ideale Flüssigkeit angeschrieben, nach Division dieser beiden Formen durcheinander auf $|\tilde w_v/\tilde w_i| = |(\lambda_i/\lambda_v)(\bar u_v/\bar u_i)|$ führt.* Hierin kann, da durch die Viskosität, von sehr großen Werten von δ/R abgesehen, die Wellenlänge λ nicht allzusehr verringert wird, $\lambda_i/\lambda_v = 1$ gesetzt werden. Die Amplituden der Quergeschwindigkeit bzw. der Auslenkung der Kanalwand nehmen somit bei gleichbleibendem Gefälle $-dp/dx$ durch den Hinzutritt der Viskosität etwa ebenso stark ab wie die mittleren Längsgeschwindigkeitsamplituden der Flüssigkeit im Kanal.

In den perilymphatischen Skalen geht infolge der Viskosität die Amplitude der Basilarmembran nur bei tieferen Frequenzen merklich zurück. Im allgemeinen ist daher die Vernachlässigung der Viskosität in der Hydrodynamik der Perilymphkanäle gerechtfertigt (vgl. jedoch Abschnitt 7).

Nach Abb. 4 ist aber auch der Viskositätseinfluß im Ductus endolymphaticus – entgegen früherer Auffassung (Ranke 1953 S. 69) – gering, wenn auch hier wiederum von tiefen Frequenzen abgesehen wird. Andererseits ist die Reißnersche Membran sehr nachgiebig (v. Békésy 1960 S. 466) so daß sie in hydrodynamischer Hinsicht nahezu wirkungslos ist. Man kann sich daher näherungsweise den Ductus endolymphaticus und die Scala vestibuli von einer einzigen Säule idealer Flüssigkeit erfüllt denken, in der dem Betrage nach ebenso große und der Richtung nach in jedem Moment

* Die vertikalen Striche bedeuten die Beträge der in allgemeinen komplexen Größen. Das Zeichen ~ bedeutet Mittelwertbildung über den Umfang U

entgegengesetzte Längsströmungen wie in der Scala tympani auftreten. Die Aufbauten der Basilarmembran stehen daher ebenso wie die der Scala tympani zugewandte Seite der Basilarmembran unter der vollen Einwirkung der perilymphatischen Drücke

Ist nun zu einem bestimmten Zeitpunkt an einem Ort im Ductus endolymphaticus ein Druckberg dann ist eine halbe Wellenlänge spitzenwärts davon ein Drucktal und nach einer weiteren halben Wellenlänge wiederum ein Druckberg Die Flüssigkeiten in den Kanalen der Aufbauten der Basilarmembran müssen daher unter der Einwirkung dieser Druckverteilung ebenso in Langsbewegungen versetzt werden wie etwa die Flüssigkeit in einem Schlauch der an zwei voneinander verschiedenen Punkten gleichzeitig gequetscht wird womit gemaß der Kontinuitatsgleichung entsprechende Wandbewegungen verbunden sein mussen

Derartige Flüssigkeitsbewegungen werden jedoch nicht eintreten wenn eine (beide) der folgenden zwei Bedingungen verwirklicht ist (sind)

a) Die *Kanalwände* (*Wande des Schlauches*) *sind so steif daß sie* durch den von außen angreifenden Flüssigkeitsdruck nicht deformiert werden können Diese Bedingung trifft in der Schnecke soweit ersichtlich vermutlich nicht zu da gemäß den Messungen v Békesys die Aufbauten der Basilarmembran eine viel geringere Steifigkeit aufweisen als die Basilarmembran selbst

b) Die Flüssigkeit in den Kanälen setzt infolge der Viskositat einer Verschiebung hohen Widerstand entgegen so daß sie scheinbar erstarrt ist Dieser Fall tritt wie Abb 4 lehrt bei hohen Werten von δ/R_k also in den ganz engen Kanälen (Tunnelraume Nuelscher Raum) bei nicht zu hohen Frequenzen tatsächlich ein

Unter der Bedingung b) (Versteifung auf Grund der Viskosität der Kanalfullung) bzw unter der bei sehr engen Kanälen immmerhin nicht ganz auszuschließenden Bedingung a) (Versteifung auf Grund der Materialeigenschaften der Kanalwand) ist die mittlere Langsgeschwindigkeitsamplitude $\bar{u}$ nahezu Null und damit die Größe der Querschnittsfläche des Kanals zeitlich nahezu unveranderlich Die Wände derartiger Kanale mussen dann nach der Kontinuitätsgleichung angenähert mit den von der Basilarmembranschwingung aufgezwungenen gegebenenfalls durch die elastischen Kräfte des Systems modifizierten Amplituden schwingen

In allen anderen Fällen sind Langsströmungen der Flussigkeit und damit zeitliche Veränderungen der Kanalquerschnitte im Takt der erregenden Frequenz zu erwarten Dies bedeutet daß wenn einem Punkt des Kanalumfanges eine der Basilarmembranschwingung entsprechende Auslenkung aufgepragt ist, ein anderer Punkt eine von dieser Auslenkung der Amplitude Phase und auch dem Verlauf über x nach verschiedene Bewegung ausfuhrt die sich der *benachbarten Zellstruktur mitteilen wird Es ist möglich daß* hier ein Zusammenhang mit den von v Békésy beobachteten Schwingungen in den Aufbauten der Basilarmembran in einer in bezug auf die Schneckenachse radialen Richtung besteht (v Békésy 1900 S 406)

Ein Beispiel für einen derartigen schwingungsfähigen Kanal bietet der Canalis sp ralis, bei dem gemäß Abb 4 auch noch bei tieferen Frequenzen die Flussigkeit eine gewisse Längsverschieblichkeit aufweist

6 *Tangentialspannungen*

Eine weitere bisher nicht beachtete Auswirkung der Viskosität ist die mit der erregenden Frequenz sinusförmig wechselnde Schubkraft, die in Längs richtung des Kanals an den Wänden angreift. Wie die gemäß dem Anhang ermittelte Kurve für die Amplitude der mittleren Schubspannung an der Wand τ (bezogen auf die Maximalamplitude τ_{max} bei $\delta/R > 1$) in Abb 4 zeigt, ist die Schubspannung im Grenzschichtbereich noch gering, wächst aber dann ziemlich rasch an Je mehr durch den Viskositätseinfluß die Querauslenkung der Kanalwände abnimmt (vgl die Kurve $|\Omega/\Omega|$ und Abschnitt 5), um so mehr nimmt die Tangentialspannung zu die den starken Abfall der Längsgeschwindigkeit u in der Nähe der Kanalwände (vgl. Abb 2 und 3) durch Mitnahme der Kanalwände in tangentialer Richtung rück gängig zu machen sucht. Dieser Tendenz steht freilich ein tangentialer Schwingungswiderstand der Kanalwand entgegen, der im allgemeinen ziem lich groß sein wird weil sich in der Ebene der Wand ein Abschnitt gegen den n der x Richtung benachbarten Abschnitt abstützt. Es ist aber auch nicht so sehr an eine Mitnahme der gesamten Kanalwand zu denken sondern nur an ein teilweises Mitflottieren von besonders nachgiebigen der Strömung stark ausgesetzten Elementen der Wandoberfläche v Békésy (1960 S 496) hat Schwingungen der Hensenschen Zellen und anliegender Teile beobach tet, die in der Schneckenlängsrichtung x also in Richtung der Schubspan nungen, erf lgten. Es ist durchaus möglich daß derartige Schwingungen durch on de Viskos tät hervorgerufene Schubspannungen angeregt werden

Well ndampfung

Di Wellendampfung infolge der Viskosität laßt sich aus folgendem Bei piel abschätzen.

Ein Schlauch aus elastischem dämpfungsfreiem Material sei mit reibungs freier Fl ssigkeit gefullt und von einem Ende her mit einem Sinuston erregt, w bei sich laufende Wellen mit konstanter Druckamplitude ausbilden mögen die Wellenlange sei λ Wird dieser Schlauch nunmehr mit viskoser Flussig keit gefullt, dann nimmt die Druckamplitude in Fortpflanzungsrichtung ab Es sei Δ diejenige Laufstrecke der Welle (gemessen in Vielfachen der Wellenlange λ) nach der die Druckamplitude auf die Halfte abgeklungen ist (H lbw rt l nge) Aus dem Anhang und z. B aus Fletcher (19 1) nd Skudrzyk (1954) kann abgeleitet werden $\approx 0{,}22\ R_s/\delta$ fur die Grenz schichtstromung und $\approx 0\ 0\ 8\ R_s/\delta$ fur die schleichende Bewegung. Fur alle Wert v n δ/R ist in Abb dargestellt

Als Mittelwert der Druckwellenlange in der Schnecke (die Wellenlänge nimmt bekanntlich v n hohen Werten am Steigbügel bis zum spitzenseitigen Ende der Erregung stetig ab) wird man etwa $\lambda = 3$ cm annehmen können

Für die Perilymphkanäle ist nach Abb. 1 und 5 bei 800 Hz = 7 und damit $\lambda = 21$ cm. Die Halbwertslänge beträgt sohin bei dieser und noch mehr bei höherer Frequenz ein Mehrfaches der tatsächlichen Lauflänge der Welle. Bei 50 Hz hingegen ist etwa 0,75 und $\lambda = 2,25$ cm. Bei dieser Frequenz gehen also vom Steigbügel bis zum Amplitudenmaximum (rund 3 cm vom Steigbügel entfernt) mehr als 50 % der Druckamplitude durch die Viskosität der Perilymphe allein verloren, was früheren Überlegungen von Jung (1940) und Ranke (1953, S. 99) entspricht.

Im Abschnitt 5 wurde vorerst zur besseren Übersicht $dp_v/dx = dp_i/dx =$ konst. vorausgesetzt, wobei bereits durch die Erhöhung des Längswiderstandes der Flüssigkeit infolge der Viskosität eine Verringerung der Trennwandamplitude auftritt. Nach obigem nimmt aber durch die Viskosität auch die Druckamplitude während des Fortschreitens der Welle ab, so daß in Wirklichkeit an einer bestimmten Stelle das Druckgefälle $-dp/dx$ bei viskoser Flüssigkeit kleiner als bei idealer sein muß, woraus eine weitere Verringerung der Trennwandamplitude resultiert.

Bei den übrigen Schneckenkanälen wird die Energie nicht von einem Ende des Kanals, sondern vom Kanalumfang her (über die gesamte Länge des Erregungsgebietes) zugeführt. Soll hiebei über eine Länge Δx die Druckamplitude gleich groß gehalten werden, dann muß über diese Länge ebensoviel Energie zugeführt werden, als bei einem lediglich von seinem Ende her mit Energie versorgten Kanal über die Länge Δx unter sonst gleichen Umständen an Energie verzehrt wird. Diese zuzuführende Energie steigt, wie aus Abb. 5 abgeschätzt werden kann, mit zunehmendem δ/R_k außerordentlich stark an (je kleiner die Halbwertslänge, um so mehr Energie muß zugeführt werden). Woferne also überhaupt Druckwellen in den engen Kanälen ablaufen — und dies ist nach Abschnitt 5 zumindest für einen Teil dieser Kanäle vorauszusetzen — ziehen diese Druckwellen einen erheblichen Energietransport von der Perilymphe quer durch die verschiedenen Kanalwände in die übrigen Innenohrflüssigkeiten mit sich.

Die starke Dämpfung der Wellen im Innenohr hat somit drei Ursachen, nämlich a) die Viskosität der Perilymphe, die nur bei tiefen Frequenzen ins *Gewicht fällt, b) die Viskosität der Flüssigkeiten in den inneren Schneckenkanälen,* deren Auswirkung sicherlich schon bei den höchsten Frequenzen einsetzt (vgl. Abb. 1, 4 und 5) und c) die *Materialdämpfung* in den elastischen schwingenden Bauteilen, die zum Teil ebenfalls eine Folge der Viskosität der Zellinhalte ist.

8. *Erhöhung der Viskosität*

Die bisherigen Überlegungen beziehen sich auf eine Flüssigkeit von der Viskosität des Wassers bei 37° C. Nun können aber spätere Messungen doch noch höhere Werte für die Viskosität in einzelnen Kanälen ergeben. Einbauten, z. B. die den inneren Tunnelraum durchziehenden Nervenfasern, sind als Strömungshindernis zu werten und wirken wie eine Erhöhung der Visko-

sität; pathologische Ursachen können zu einer Vergrößerung der Viskosität führen.

Eine Erhöhung der Viskosität auf das n fache erhöht wegen $\delta = \sqrt{\eta/\varrho f}$ die Kenngröße δ/R auf das $\sqrt{n}$ fache und hat im übrigen dieselbe Wirkung wie eine Verringerung der Frequenz auf das $1/n$ fache[1]. Diese Erhöhung bedeutet in Abb. 1 eine Verschiebung der Geraden des Nomogrammes in Richtung der Abszissenachse nach rechts um eine Strecke, die aus der in der linken unteren Ecke von Abb. 1 eingezeichneten Skala abgelesen werden kann. Die Auswirkungen sind hiebei je nach der Größe von δ/R_k unterschiedlich und können keinesfalls, wie dies von Ranke (1953) S. 69 versucht worden ist, aus einem allgemeinen Modellgesetz abgeleitet werden. Betrachtet man z. B. die Änderung des Verhältnisses $|\bar{u}_s/\bar{u}|$ bei 800 Hz infolge einer Erhöhung der Viskosität auf das Dreifache, dann ändert sich für die perilymphatischen Skalen so gut wie nichts, weil im Bereich der Grenzschichtströmung die Kurve für $|\bar{u}_s/\bar{u}|$ sehr flach verläuft. Für den Canalis spiralis hingegen tritt wegen des steilen Abfalles der Kurve für $|\bar{u}_s/\bar{u}|$ eine erhebliche Verringerung ein. Die größten Änderungen sind im Übergangsbereich zwischen Grenzschichtströmung und schleichender Bewegung zu erwarten; auch die Kurve für in Abb. 5 zeigt im Übergangsbereich einen steileren Abfall als in den beiden Grenzbereichen.

Eine Vergrößerung der Viskosität wird allgemein gesprochen a) die Dämpfung (Energieschluckung) erhöhen (Abschnitt 7), was eine Verringerung der Auslenkungsamplituden und eine Verschiebung der Stelle des Trennwandamplitudenmaximums in Richtung auf die Schneckenbasis zur Folge hat, b) die Unterschiede in den Querbewegungen der einzelnen Bauteile herabsetzen (Abschnitt 5) und c) die Schubspannungen und damit die Tendenz zur Längsbewegung einzelner Teile der Kanalwandung erhöhen (Abschnitt 6).

ANHANG. NÄHERUNGSLÖSUNGEN TANGENTIALSPANNUNGEN

Die Gleichung für die Bewegung einer viskosen Flüssigkeit in der Längsrichtung x eines Kanals lautet bei sinusförmiger Erregung mit der Kreisfrequenz $\omega = 2\pi f$

$$-\frac{\partial p}{\partial x} = i\omega\varrho u - \eta\frac{\partial^2 u}{\partial x^2} - \eta\Delta^{(2)} u \tag{1}$$

worin $\Delta^{(2)}$ den Laplaceschen Operator für die Ebene des Querschnittes S bedeutet und $i = \sqrt{-1}$ ist. Nach Mittelwertbildung über den Querschnitt S wird (1) zu

$$-\frac{d\bar{p}}{dx} = i\omega\varrho\bar{u} - \eta\frac{d^2\bar{u}}{dx^2} - \eta\frac{1}{S}\iint\Delta^{(2)} u\, dS \tag{2}$$

[1] Bei Modellversuchen ist selbst ersichtlich die Kennzahl δ/R genau einzuhalten, was bei Vergrößerung von R durch Herabsetzung der Frequenz und/oder Vergrößerung der Viskosität leicht zu erreichen ist.

Bei idealer Flüssigkeit ($\nu = 0$) reduziert sich (1) bzw (2) auf

$$-\frac{\partial p}{\partial x} = i\omega\varrho u_i \qquad -\frac{d\bar{p}}{dx} = i\omega\varrho \bar{u}_i. \tag{3}$$

Im vorliegenden Fall sind drei Näherungslösungen von (1) und (2) von Interesse nämlich

a) die Grenzschichtlösung (vgl Cremer 1950 S 298 und Abb 2)

$$u = u_i(P)\,[1 - e^{-(1-i)s/\delta}] \tag{4}$$

$$\bar{u} = \bar{u}_i[1 - (1-i)\delta/R_h] = \bar{u}_i[1 - (1-i)\delta/R_h] \tag{5}$$

($u_i(P)$ = Amplitude der Längsgeschwindigkeit bei idealer Flüssigkeit in einem Punkt P der Kanalwand s von der Kanalwand in das Innere weisende Koordinate $\bar{u}_i$ über den Kanalumfang gemittelter Wert der Längsgeschwindigkeit wobei für die Schneckenkanäle $\bar{u}_i = \bar{u}_i$ gesetzt werden kann)

Diese Lösung gilt wie Einsetzen in (1) bzw (2) bei Annahme einer permanenten Welle zeigt unter den Bedingungen $\delta/R_h < 1$ $2\pi\delta/\lambda < 1$ Da der Kleinstwert von λ nach C v Békésy (1960) S 460 etwa bei 0 25 0 5 cm liegt sind diese Bedingungen, wie mit Tabelle 1 und 2 folgt etwa für $\delta/R_h \leq 0.3$ gut erfüllt.

b) Die Lösung für den Kanal mit Kreisquerschnitt (vgl Schlichting 1958 S 215)

$$u = \bar{u}_i\{1 - J_0[(1-i)r/\delta]/J_0[(1-i)R/\delta]\} \tag{6}$$

$$\bar{u} = \bar{u}_i\{1 - 2\delta J_1[(1-i)R/\delta]/(1-i)RJ_0[(1-i)R/\delta]\} \tag{7}$$

(R Radius des Kreisquerschnittes r in radialer Richtung laufende Koordinate J_0, J_1 Besselfunktion nullter bzw erster Ordnung) Diese Lösung gilt unter der Bedingung $2\pi R/\lambda > 1$ für beliebige Verhältnisse δ/R

c) Die Lösung fur die schleichende Bewegung (der Grenzfall von b) für $R/\delta \to 0$) bei kreisförmigem Querschnitt (vgl Berker 1963 S 69)

$$u = i\bar{u}\,(R^2 - r^2)/2\delta \tag{8}$$

$$\bar{u} = i\bar{u}_i(R/2\delta) \tag{9}$$

(9) gilt jedoch auch für von der Kreisform abweichende Querschnitte wofern statt R ein gleichwertiger Radius R eingeführt wird der sich aus der Forderung bestimmt daß für den Kreisquerschnitt und für den von der Kreisform abweichenden Querschnitt $\bar{u}_i$ und $\bar{u}$ gleich groß sein sollen Idealisiert man die Querschnittsformen der Schneckenkanäle zu Dreiecken bzw Rechtecken bzw Ellipsen dann kann man aus den bekannten Lösungen für diese Querschnittsformen (vgl Berker 1963 S 69 f) R berechnen es zeigt sich daß für samtliche Schneckenkanäle $1 \leq R$ R $\leq 1\,075$ ist, so daß statt (9) näherungsweise

$$\bar{u} = i\bar{u}\,(R_h/2\delta) \tag{10}$$

geschrieben werden kann Die Bedingungen fur die Gultigkeit von (9) bzw (10) sind $R_h/2\delta < 1$ und $2\pi R_h/\lambda < 1$ sie können für δ $R_h \geq 1$ als erfullt angesehen werden Wegen des geringen Unterschiedes zwischen R und R_h kann auch in (7) R durch R_h ersetzt und die so gewonnene Formel näherungsweise für alle Schneckenkanäle angewendet werden.

Bestimmt man aus dem Newtonschen Ansatz [illegible] $= \eta(\partial u_x/\partial s)$ die über den Umfang gemittelte Amplitude [illegible] der Tangentialspannung aus (5), (7) und (9), dann erhält man für die obigen drei Fälle a) bis c):

[illegible]) $\tau = -(1+i)\,\omega\varrho\Pi_0\delta/2 \approx -(1 [illegible] i)\,\omega\varrho\Pi_0\delta/2,$ (Grenzschichtlösung),

b) [illegible] $(1-i)\,\omega\varrho\Pi_0\delta J$ [illegible] $[(1 [illegible] i)\,R/\delta]/2J$ [illegible] $[(1-i)\,R/\delta]$ (Kreisrohr)

[illegible]) [illegible] $i\omega\varrho\Pi\ R_h/2 - \frac{dp}{dx} R$ [illegible] $/2$ (schleichende Bewegung, die Formel gilt für beliebiges R [illegible] genau).

Die Bedingungen für die Gültigkeit sind im wesentlichen dieselben wie die oben zu [illegible]), b) [illegible]nd [illegible]) angegebenen.

SUMMARY

The effect of viscosity on wave propagation inside the fluid-filled channels of the cochlea (peri- and endolymphatic scales, canalis spiralis, etc.) is represented as a f[illegible]nction of one single characteristic (thickness of the acoustic boundary layer/hydra[illegible]lic radius of the channel). This effect increases, when the frequency and/o[illegible] the area of the section of the channels decrease [illegible] resulting in a growing resistance of the fluid to longitudinal movement and, consequently [illegible] in a diminution of the excursions [illegible]f the walls [illegible]f the channels. Furthermore a longitudinal shearing force is generated, causing longitudinal movement [illegible] of the walls. The wave attenuation due to viscosity and the consequences of a change in the magnitude of the viscosity are discussed.

LITERATUR

Békésy, G. [illegible] 1960: *Experiments in Hearing* McGraw Hill, New York.

B[illegible], R. 1963 Intégration des équations du mouvement d'un fluide visqueux. In: *Handbuch der Physik* Bd. VIII/2. Springer Verlag, Berlin.

C[illegible], L., 1950 Die wissenschaftlichen Grundlagen der Raumakustik. In: W[illegible] *[illegible] Raumakustik* Bd. III. Verlag Hirzel, Leipzig.

[illegible], J. und Lax, E., 19[illegible] *Taschenbuch für Chemiker und Physiker* Springer Verlag, Berlin.

Fletcher, H. 1951 On the dynamics of the Cochlea, *J. Acoust. Soc. Amer.* *[illegible]*, 637

J[illegible] H. 19[illegible]: Untersuchungen zu den Theorien des Hörens. *Akust. Z.*, *4*, 2[illegible].

Kr[illegible], M., 1953 *Probleme der Ohrphysiologie und neue Lösungsversuche* Springer Verlag, Wien.

N[illegible] K. 1950 Die Basilarmembran des Menschen und ihr Verankerungssystem. *Z. Anat. Entwicklungsgesch.*, *114* 539

R[illegible], O. F. 1933 *Physiologie des Gehörs*. Springer Verlag, Berlin.

R[illegible], S. (Hrsg.) 196[illegible]. *Biochemie des Gehörorgans* G. Thieme, Stuttgart.

Schlichting, H. 1958 *Grenzschichttheorie* 3. Aufl. G. Braun, Karlsruhe

S[illegible], E., 1931 *Die Grundlagen der Akustik*. Springer Verlag, Wien.

Währingerstr. 1[illegible] 1[illegible] Wien, Österreich

Eingegangen am [illegible] Januar 1967

Acta oto-laryngologica 64 148–158 (1967)

AUTORADIOGRAPHISCHE UNTERSUCHUNGEN ZUM STOFFWECHSEL DES DUCTUS UND SACCUS ENDOLYMPHATICUS

E KOBURG J HAUBRICH und B KERNBACH
Düsseldorf und Göttingen Deutschland

Aus der Universitäts Klinik für Hals Nasen-Ohrenkrankheiten (Direktor Prof A Meyer zum Gottesberge) Düsseldorf und der Universitäts Klinik und Poliklinik für Hals Nasen-Ohrenkrankheiten (Direktor Prof A Miehlke) Göttingen

Der Eiweißstoffwechsel des Saccus endolymphaticus (S e) wurde autoradiographisch mit H Tyrosin untersucht. Es zeigt sich daß in den Zellen der Pars intermedia des S e der höchste Eiweißumsatz vorliegt der zum Ductus endolymphaticus hin stark abfällt. Der Eiweißumsatz im subepithelialen Gewebe der Pars intermedia liegt gleichfalls recht hoch. Es werden verschiedene Strukturen im gleichen Autoradiogramm miteinander verglichen die Eiweißsyntheserate der Pars intermedia liegt höher als in der Stria vascularis und im Limbum semilunatum und niedriger als in den Ganglienzellen des Ganglion spirale

Die intrasacculären Zellen lassen sich nach der Größe ihres Eiweißumsatzes in funktionsfähige und regressiv veränderte Zellen unterteilen Die ersteren bestehen aus Makro- und Mikrophagen (Histiocyten Leukocyten) die letzteren aus abgestoßenen Saccusepithelien

Außerdem findet sich in der Saccuslichtung kondensiertes Eiweiß, welches nach einstündigem Versuchsintervall bereits eine Schwärzung erkennen läßt Dieser Befund spricht für eine beträchtliche Wanderungsgeschwindigkeit des Eiweißes, wenn es in der Cochlea sezerniert worden wäre Anderfalls könnte es von einem saccusnahen Sekretionsort herstammen

Nach den Ergebnissen aus der Literatur und den hier vorgelegten autoradiographischen Befunden kann man im Saccus zwei phagocytierende Systeme annehmen 1 die intrasacculären Zellen die aus der Endolymphe gröbere Partikel aufzunehmen vermögen und 2 das Saccusepithel der Pars intermedia, das die Endolymphe oder wenigstens einen Teil derselben resorbiert.

Den inzwischen zahlreichen überwiegend morphologischen Untersuchungen am Ductus und Saccus endolymphaticus stehen verschiedene teilweise widersprechende Deutungsversuche über die Funktion dieser Struktureinheit im Innenohr gegenüber Das ist leicht zu verstehen, da sich aus rein morphologischen Ergebnissen nur mit Einschränkung Rückschlüsse auf die mögliche Funktion von Geweben oder Organen ziehen lassen

Als Funktion des Saccus endolymphaticus werden Sekretion (Seymour

Herrn Professor D med F Zöllner zum 65 Geburtstag gewidmet.

1964 v. Egmond & Brinkman, 1956) Resorption von Endolymphe (Guild, 1927 Mygind, 1948 Altmann & Waltner 1950 Engström & Hjorth, 1950 Arnvig, 1951 Saxén, 1951 Lundquist, Kimura & Wersäll, 1964 a Lundquist, 1965 u. a.) oder beide Vorgänge nebeneinander diskutiert, ohne daß bisher eine endgültige Entscheidung möglich geworden wäre. In jüngster Zeit hat Lundquist (1965) durch seine ausführlichen elektronenoptischen Untersuchungen einen wichtigen Beitrag zum Problem der Saccusfunktion gegeben: nach dem feinstrukturellen Aufbau der Saccusepithelien und der besonderen Struktur der Gefäßendothelien im subepithelialen Gewebe liegen Resorption, Pinocytose und auch Phagocytose durchaus im Bereich der Leistungsbreite dieser Zellen. Ebenso wird auch den sog. intrasacculären Zellen eine wichtige Funktion im Rahmen der Endolymphresorption beigemessen. Diese Annahme wird von Guild (1927), Engström & Hjorth (1950) und Lundquist (1965) u. a. durch Experimente bestätigt, bei denen Fremdpartikel in den Ductus cochlearis eingebracht wurden, die dann später in den intrasacculären Zellen und den Saccusepithelien auftauchten.

Während in den angeführten Versuchen meistens nur ein momentaner Funktionszustand der Saccusepithelien und der intrasacculären Zellen erfaßt werden kann, gewährt die von uns angewandte Methode der Autoradiographie einen Einblick in die Stoffwechseldynamik der einzelnen Zellen. Ausgehend von einer Bestimmung des Eiweißstoffwechsels der Saccusepithelien und des Saccusinhaltes soll deren mögliche Funktion diskutiert und mit den vorliegenden Ergebnissen der Literatur verglichen werden.

MATERIAL UND METHODE

Meerschweinchen beiderlei Geschlechts im Gewicht von 210 bis 310 g wurden 10 mC H^3-Tyrosin[1] von hoher spezifischer Aktivität intraperitoneal appliziert. Eine Stunde nach der Injektion erfolgt die Tötung der Tiere durch Dekapitation im leichten Ätherrausch. Nach der Trepanation der Bulla und Entfernung des Steigbügels Fixierung in 6%igem Formalin. Entkalkung in einer Lösung von HCl, AlCl und Ameisensäure. Nach Paraffineinbettung 5 μ dicke Schnitt parallel zum Modiolus, wobei Wert darauf gelegt wurde, daß der Flocculus des Kleinhirns und der Sinus sigmoideus mit angeschnitten wurden. Die Autoradiogramme (ARG) wurden mittels der Stripping Film Method hergestellt und 10 bis 20 Tage exponiert. Anschließend erfolgte die Färbung der Schnitte durch den Film. Die Auswertung der ARG erfolgte durch Auszählung der Silberkörner (SK) über verschiedenen Arealen von mehr ten 1600 μ^2, wobei verschiedene Bereiche miteinander verglichen wurden. Die Silberkornzahlen (SKZ) wurden in einer Tabelle zusammengestellt.

[1] Prof. Dr. W. Maurer und Dr. Dr. K. Hempel, Instit. f. Med. Isotopenforschung der Universität Köln, danken wir für die Überlassung des H^3-Tyrosin.

TABELLE 1 *Relative Silberkorndichten als Ausdruck des Eiweißstoffwechsels in verschiedenen Arealen des Saccus endolymphaticus in Vergleich zu anderen Geweben des Innenohres*

Ganglienzellen des Ganglion spirale cochleae = 100

Gewebe	SK/μ	Relative Eiweißumsatzrate %
Saccus endolymphaticus		
Pars proximalis	0,5	51
Pars intermedia	0,8	87
Pars distalis	0 6	68
Ductus endolymphaticus	0,3	35
Subepitheliales Gewebe der Pars intermedia		
a) Bindegewebszellen	0,5	58
b) Interzelluläres Geweb		
des Ductus endolymphaticus	0 3	35
a) Bindegewebszellen	0 3	36
b) Interzelluläres Gewebe	0 1	14
Plexus cochlearis	0,8	91
Stria vascularis	0 6	65
Planum semilunatum	0 4	45
Ganglienzellen des Ganglion spirale cochleae	0,9	100

ERGEBNISSE

1 *Ductus und Saccus endolymphaticus*

Bei der Auswertung unserer Untersuchungen legten wir die von Guild (1927) erstmalig angegebene Aufteilung des Saccus endolymphaticus (S. e.) in drei Zonen zugrunde Pars proximalis Pars intermedia und Pars distalis Diese drei Saccusabschnitte können bei rein anatomischer Orientierung aufgrund ihrer unterschiedlichen Epitheldifferenzierung abgegrenzt werden Interessanterweise entsprechen dieser Einteilung in etwa die Unterschiede des autoradiographischen Schwärzungsgrades, d. h., der Größe des Eiweißstoffwechsels der Zellen (s. Tab)

Wie aus Abb 1 zu entnehmen ist, zeigt das flache manchmal leicht kubische Epithel des Ductus endolymphaticus (D e) nur eine sehr geringe Schwärzung In der sich anschließenden Pars proximalis des S e findet man eine deutlich stärkere Schwärzung Am dichtesten liegen die SK über dem häufig papillär vorgewolbten hohen Epithel der Pars intermedia (Abb 2 und 3) welches durch seine kraftige Basophilie ausgezeichnet ist Die Pars distalis ist in den einzelnen ARG nicht immer gut abgrenzbar doch hat man den Eindruck, daß sie einen der Pars intermedia annahernd vergleichbaren Eiweißumsatz hat

Eine sichere autoradiographische Unterscheidung zwischen den sog hel

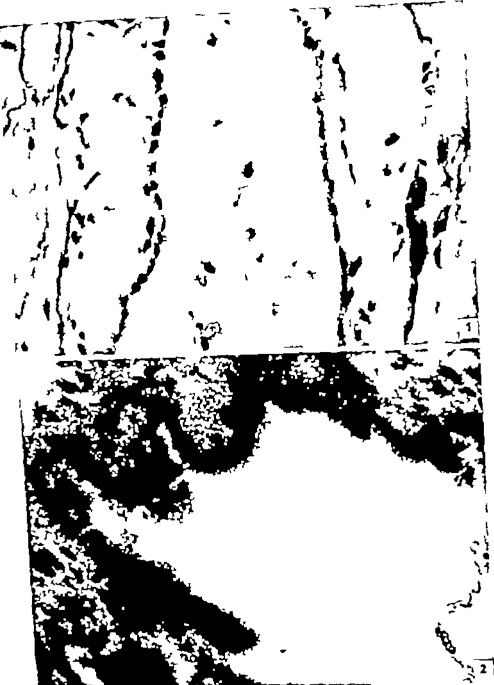


Abb. 1. Autoradiogramm der Pars proximalis des Saccus endolymphaticus des Meerschweinchens eine Stunde nach Gabe von H³-Tyrosin. Strippingfilm, HE-Färbung, 300fache Vergrößerung. Nur wenig Silberkörner als Maßstab der Größe des Eiweißumsatzes über dem Epithel und dem subepithelialen Gewebe. Über den der Lichtung liegenden Zellen erkennt man nur sehr wenig Silberkörner.

Abb. 2. Autoradiogramm der Pars intermedia des Saccus endolymphaticus des Meerschweinchens eine Stunde nach Gabe von H³-Tyrosin. Strippingfilm, HE-Färbung, 300fache Vergrößerung. Zottenartige Vorwölbung des Epithels, das einen sehr hohen Eiweißumsatz, ausgedrückt durch eine hohe Silberkorndichte, erkennen läßt.

TABELLE 1 *Relative Silberkorndichten als Ausdruck des Eiweißstoffwechsels in verschiedenen Arealen des Saccus endolymphaticus in Vergleich zu anderen Geweben des Innenohres*

Ganglienzellen des Ganglion spirale cochleae = 100

Gewebe	SKZ/μ	Relative Eiweißumsatzrate %
Saccus endolymphaticus		
Pars proximalis	0,5	54
Pars intermedia	0,8	87
Pars distalis	0 6	68
Ductus endolymphaticus	0,3	35
Subepitheliales Gewebe der Pars intermedia		
a) Bindegewebszellen	0,5	58
b) Interzelluläres Gewebe des Ductus end lymphaticus	0,3	33
a) Bindegew bszellen	0,3	36
b) Interzelluläres Gewebe	0,1	14
Plexus cochlearis	0 8	91
Stria vascularis	0,6	65
Planum semilunatum	0,4	45
Ganglienzellen des Ganglion spirale cochl ae	0,9	100

ERGEBNISSE

1 *Ductus und Saccus endolymphaticus*

Bei der Auswertung unserer Untersuchungen legten wir die von Guild (1927) erstmalig angegebene Aufteilung des Saccus endolymphaticus (S. e.) in drei Zonen zugrunde Pars proximalis, Pars intermedia und Pars distalis. Diese drei Saccusabschnitte konnen bei rein anatomischer Orientierung aufgrund ihrer unterschiedlichen Epitheldifferenzierung abgegrenzt werden Interessanterweise entsprechen dieser Einteilung in etwa die Unterschiede des autoradiographischen Schwarzungsgrades, d h der Größe des Eiweißstoffwechsels der Zellen (s. Tab)

Wie aus Abb 1 zu entnehmen ist zeigt das flache manchmal leicht kubische Epithel des Ductus endolymphaticus (D e) nur eine sehr geringe Schwarzung In der sich anschließenden Pars proximalis des S e findet man eine deutlich starkere Schwärzung Am dichtesten liegen die SK über dem haufig papillär vorgewölbten, hohen Epithel der Pars intermedia (Abb 2 und 3) welches durch seine kräftige Basophilie ausgezeichnet ist Die Pars distalis ist in den einzelnen ARG nicht immer gut abgrenzbar doch hat man den Eindruck, daß sie einen der Pars intermedia annähernd vergleichbaren Eiweißumsatz hat

Eine sichere autoradiographische Unterscheidung zwischen den sog hel

len und dunklen Zellen (Lundquist, Kimura & Wersäll, 1964 b) im S. e. kann nicht erzielt werden Andererseits ist auch die lichtmikroskopische Differenzierung der beiden Zellarten schwierig und meistens nicht mit Sicherheit durchführbar

2 Subepitheliales Gewebe

Im subepithelialen Bereich der Pars intermedia des S e. ist einmal die Anzahl der Bindegewebszellen deutlich höher als an entsprechender Stelle im D e zum anderen haben die Bindegewebszellen der Pars intermedia eine fast doppelt so große Anzahl von Sk wie die des D e Auch die Größe der Zellen variiert beträchtlich in den verschiedenen Abschnitten zugunsten der intermediären Portion Ähnliche Unterschiede im Schwärzungsgrad lassen sich auch im interzellulären Gewebe des subepithelialen Bereiches nachw isen die Pars intermedia weist eine gut doppit so h he SK Dichte wie der Ductusabschnitt auf

3. Inhalt des Saccus endolymphaticus

In der Lichtung des S. e sieht man zahlreiche frei flottierende Zellen von oft bizarrer Gestalt (Abb. 3 und 4) An einigen Stellen stehen sie in losem Kontakt mit dem auskleidenden Saccusepithel oft liegen sie mitten in der Lichtung Auffallend ist die Lokalisation der Zellen sie finden sich überwiegend im intermediären Teil des Saccus.

Um inen Überblick hinsichtlich des Funktionszustandes der intrasac culären Zellen zu erhalten, wurden die Zellen nach ihrem Schwärzungsgrad eingeteilt Wir unterscheiden a) stärker geschwärzte b) schwach geschwärzt c) ungeschwärzte Zellen

In der rsten Kategorie finden sich häufig Zellen von beträchtlicher Große mit einem Durchmesser bis zu 25 μ deren Cytoplasma oft sehr hell erscheint. Eine mäßige Basophilie besteht manchmal perinucleär Licht mikroskopisch lassen sich kleine Vakuolen im Cytoplasma wahrnehmen, die m n hmal mit einem feingranulären Material angefüllt sind. Der chroma tindicht le fl etwas lebelförmig ausgezogene Kern kann an den Rand der Zelle gedrängt sein Zwei- und dreikernige Zellen sind nicht selten anzutreffen, K rnteilu gsfiguren la sen sich nur ganz vereinzelt beobachten Bei der Beurt ilung des Schwärzungsgrades muß die große Fläche der Zellen berücksichtigt werden. Eine pro Flächeneinheit nur geringe Sk-Dichte l ßt trotzdem auf einen großen zellulären Eiweißumsatz schließen wenn diese Zelle sehr groß ist

In die zweit Gruppe lassen ich die immer wieder anzutreffenden Leukocyten (die ohnehin einen gering n Eiweißstoffwechsel haben) und anscheinend gerade abgest ßene Saccusepithelien mit einem noch geringerem Eiweißst ffwechsel einordnen. Letztere Zellen weisen bereits regressi e Merkmale wie beginnende Homogeni ierung ode diffuse Einwässerung (hydropische Schwellung) des Cytoplasma ls Ausdruck einer energetischen



Abb 3 Aut radiogramm d Pars i t rmedia de Saccu d i mph t cus des Meer schweinch n In Stu d na h G be n H³ Tyro l Zahl i h Silbe k rn uber dem zyli drische Saccusepith l In der S een lichtu g k d n ertes Eiw iß it e nig frei fl ttierend n Z ll Li ks i zw kernig Z ll Über d n Eiw ißkoa rvat n und d n freien Zellen rk t man zahlrei he Silbe körner

Abb 4 Autoradiogramm des Saccu l haltes des Meerschwe h n In St d nach Gabe o H³ Tyrosl Strippingfilm, HE Farbu g, 300fa h Vergroßeru g Man erke t Silberkorner sow hl uber d n Eiw ißkoa rv t l auch her d n l d Licht ng liege d n Zellen

Abb. 1–4 stellen Wiedergaben n A t radi gramm n des gl iche V rsuches bei in u d dei ben Meerschw inchen und gl iche Belicht ngsz it dar Di S wärsungen (SKZ Fl neinh it) ind lso unmitt lbar ergl ichba

phagocytierende Zellen des entzündlichen Granulationsgewebes der Ratte ein sehr hoher Eiweißumsatz fand, der etwa dem von Ganglienzellen und Eiweiß sezernierenden Zellen gleichkommt. Die im subepithelialen Gewebe gemessene Aktivität liegt im intermediären Saccusteil gleichfalls höher als an entsprechender anderer Stelle was man auf die hier gelegenen „großen Zellen" der Bindegewebsreihe — Fibroblasten und Histiocyten — zurückführen kann. In Anpassung an ihren Wirkungsort finden sich diese Zellen überwiegend im subepithelialen Gewebe der intermediären Portion.

2. Zur Größe und Bedeutung des Eiweißumsat.es des ellulären Saccusinhaltes

Neben den differenzierten Saccusepithelien scheinen die frei in der Lichtu g liegenden Zellen ein weiterer wichtiger Faktor für die Funktion des S. e zu sein. Vereinfachend können wir nach der Größe der Eiweißumsatzrate zwei Zellarten unterscheiden funktionstüchtige und regressiv veränderte Zellen des RES und einige polymorphkernige Leukocyten, also einmal Zellen, die im Rahmen der Zellmauserung aus ihrem Verband eliminiert worden sind, und zum anderen "aktive" Zellen, die wir als Makro- und Mikrophagen bezeichnen möchten

Die SK Dichte über den „aktiven Zellen läßt sich zwar nicht unmittelbar mit derjenigen über den Saccusepithelien vergleichen, erreicht aber dennoch ine ansehnliche Größe wenn man zwei Dinge berücksichtigt 1 haben di Zellen eine erheblich Größe und damit eine breitere räumliche Verteilung der Eiweißsyntheserate, die SK-Dichte muß also geringer sein als bei kompakteren Zellen, und 2. beziehen sie während der kurzen Verweilzeit ihre markierte Aminosaure all in durch Diffusion aus der Endolymphe der wahre Eiweißumsatz ist also wegen des letzteren Gesichtspunktes wahrscheinlich größer als ihn die SK Dichte anzeigt

Aus den ARG läßt sich unschwer entnehmen, daß nicht alle Makrophagen eine gleiche Zellaktivität besitzen. Einige weisen eine diffuse oder vakuolige hydropische Schwellung des Cytopla mas auf was als regressives Merkmal nzusehen ist. In solchen Fäll n ist die Zahl der SK, d. h., die eingebaute Aktivität geringer

Berucksichtigt man ußerdem die reiche Fermentausstattung (Ishii, Silverstein & Balogh, 1966) und unsere autoradiographischen Ergebnisse zum Eiweißst ffwechsel der flottierenden Saccuszellen, so kann man der Ansicht n Altmann & Waltner (19.0) nur zustimmen sie glauben, daß diese Zellen zu Aufnahme on gröberen Partik ln befähigt sind, die an anderer Stell durc Resorption nicht aufgenommen werden können. Es ist bekannt, daß überwiegend die Zellen des RES eine Phagocytose durchführen, während Epithelzellen in nur geringem Maße dieser Aufgabe nachkommen. Guild (193) Engström & Hjorth (1950) Andersen (1948) v Egmond & Brinkman (1956) Lundquist (1965) u. a haben in Übereinstimmung mit dieser Auffas ung eine st rke Speicherfähigkeit für injizierte Fremdstoffe i den flottierenden Saccuszellen nachgewiesen.

Insuffizienz auf Außerdem findet man immer wieder kleinere und größere Vakuolen die oft den ganzen Zelleib ausfüllen

Als dritte Gruppe finden sich Zellen mit beginnender Nekrose die nur noch einen geringen Eiweißstoffwechsel aufweisen Ihr Cytoplasma ist homogen eosin gefärbt der Kern gekerbt oder karyorhektisch. Die in diesem Stadium befindlichen Zellen lassen sich nicht mehr mit genügender Sicherheit einem Herkunftsort zuordnen

Zwischen den zellulären Bestandteilen im Lumen des S e ist ein amorphes, homogen mit Eosin gefärbtes Material nachzuweisen, welches, seiner Struktur nach ausgefälltem Eiweiß entspricht Über diesem Eiweiß liegen ebenfalls Sk (Abb 3 und 4) Außerdem sieht man ein feinkörniges, aufgelockertes Material ohne diese intensive Färbung und ohne Sk Hier konnte es sich um Zelltrümmer handeln

BESPRECHUNG DER ERGEBNISSE

1 *Zur Große und Bedeutung des Eiweißumsatzes der Saccuswandung*

Nach den autoradiographischen Ergebnissen zeigt sich in den Epithelzellen des intermediären Saccusbereiches eine sehr hohe Eiweißneubildungsrate die zum proximalen Teil d h., zum D e hin mehr und mehr abfällt. Um die Größe des Eiweißumsatzes (EU) zu veranschaulichen, wurden in ein und demselben Autoradiogramm Strukturen miteinander verglichen und zueinander in Beziehung gesetzt Der EU im intermediären Teil des S e liegt um etwa 15–20% niedriger als der in den Ganglienzellen des Ganglion spirale höher als in der Stria vascularis und im Planum semilunatum etwa gleich hoch wie im Plexus cochlearis (Koburg 1964 Balogh & Koburg, 1965) Die Feststellung einer beträchtlichen Epithelaktivität im intermediären Teil des S e entspricht in sehr schöner Weise den von Guild (1927) u a gemachten Beobachtungen in besonderem Maße aber den Ergebnissen von Lundquist (1965) der aufgrund seiner elektronenoptischen Bilder eine lebhafte Pinocytose und Phagocytose in den Saccusepithelien annimmt

Die hohe Eiweißsyntheserate steht u E. in unmittelbarem Zusammenhang mit der Funktion der Epithelien als „phagocytierendes System" In derartigen Zellen erwartet man einen außerordentlich lebhaften Stoffwechsel wie er zur Synthese zahlreicher in Phagocyten vorhandener Enzyme notwendig ist (Lit bei Ehrich 1956) Tatsächlich ist nach Ishii Silverstein & Balogh (1966) der Enzymgehalt des Epithels im intermediären Saccusbereich hoch während an anderen Stellen des S. e eine wesentlich geringere Aktivität nachgewiesen werden kann Lediglich der Gehalt an Aminopeptidasen ist im Epithel genau so hoch wie im perisacculären Bindegewebe Als eine Parallele zu unserer Feststellung eines hohen Eiweißumsatzes können die *autoradiographischen Untersuchungen* von Niklas & Oehlert (1956) und Oehlert (1959) gelten in denen sich für verschiedene

gocytierende Systeme bestehen auf der einen Seite die Epithelien der intermediären Saccu portion, auf der anderen Seite die intrasacculären Zellen Letztere setzen sich zu einem Teil aus Histiocyten—Makrophagen und Leukocyten—Mikrophagen zusammen die aus dem subepithelialen Gewebe in das Saccuslumen immigrieren Hier finden sich in den Buchten des sich papillar vorwölbenden Epithels optimale Verhältnisse für die Phagocytose. Damit soll nicht behauptet werden, daß der S. e der einzige Resorptionsort für die Endolymphe wäre. Doch darf man ihm eine besondere Rolle für die „Endolymphreinigung" zuerkennen, wofür die in der Saccuslichtung anzutreffen den Makro- und Mikrophagen sprechen Die intrasacculären Zellen wären daher als „Vorfilter" für die im Epithel zu resorbierende Endolymphe an zu prechen

Die Arbeit wurde unterstützt durch Mittel der Deutschen Forschungsgemeinschaft. Fü t ch nische Hilfe und Herst llung der Autoradiogramme danken wir F I. Dr ka Hinterleitner

SUMMARY

I the endolymph tic sac (e s.) f guinea pigs protein metabolism was in vest gated by mean of H³-tyrosine-autoradiography As demonstrated prot in turnover was highest in the cells f the intermedi te part of the e s., but decreased con iderably towards the endolymphatic duct. In the subepith lial layer the turn er rate f prot i was also found t be rather high. By comparison with th turnover rates of the different structures of the i ner ear the e s. luded, t was shown that the sy thetic acti ity f the intermediate part of th s. was higher than that of the st ia vasculari and the planum semilunatum but lower than that f the ochlear ganglionic cells.

A cord ng t their magnitude of protein metabolism, the intrasaccular cells could be di ided into those with functional act vity and those which re obviously dying The former are identical with macrophages nd microphages (histiocytes, leucocytes) the latter w th exfoliated epithelia of the saccus.

Des des thre e llular cont nt, the lumen of the s. contain condensed protein which even 1 hour after administration f the radioacti compound w s found to be labelled. If ne assumes that the prot in was secreted in the cochlea this result ind cates a considerable peed of migration of the prote n f the endolymphat s stem. On the other ha d, th labelled protein could originate from a t adjace t to the e s.

Based on the results of other investigators a d on the autoradiographic findings reported bo e the s. seem to posses two phagocytic system (1) The intrasaccular cells, which may be able t remove cellular debri. (2) The epithelium of the intermedia part f th s. whi h may be able to resorb at least part f the endolymph.

LITERATUR

Altm F and W ters, J. G., 1950 F rther investigations on the physi logy f the l byri thine fl ids. A n. Otol 59 657

Die immer wieder auftretenden mehrkernigen Riesenzellen lassen sich als Ausdruck einer erhöhten Stoffwechselleistung dieser Zellen werten (Letterer 1951). In ähnlichem Sinne würden sich die hin und wieder wahrnehmbaren Mitosen in den freien Saccuszellen deuten lassen. Eine phagocytotische Tätigkeit der abgestoßenen Saccusepithelien ist nicht mehr zu erwarten, da eine solch differenzierte Leistung wie sie die Phagocytose darstellt, einen sehr aktiven Stoffwechsel voraussetzt. Dazu ist eine aus ihrem festen Gefüge herausgelöste Epithelzelle nicht mehr fähig. Autoradiographisch finden sich über solchen Zellen keine oder nur sehr wenige SK.

3. *Zur Größe und Bedeutung des Eiweißumsatzes des nichtcellulären Saccusinhaltes*

Die intrasacculären Zellen liegen häufig eingebettet in einem mit Eosin angefärbten, kondensierten Eiweiß. Das ist um so bemerkenswerter als die Endolymphe sich normalerweise mit Eosin nicht anfärben läßt (Rauch, 1964; Schuknecht & McNeill, 1966). In letzter Zeit hat Silverstein (1965) in biochemischen Untersuchungen ebenfalls einen erhöhten Eiweißgehalt im S. e. des Meerschweinchens festgestellt. Er nimmt eine hohe Wasserresorption durch die Saccusepithelien an, wodurch es zu dieser Eiweißkonzentration kommt.

In unseren Versuchen zeigen sich bereits eine Stunde nach der Injektion einzelne SK über dem Saccuseiweiß. Das kann folgendes bedeuten: Sollte das Eiweiß aus der Cochlea stammen, müßte man eine beträchtliche Wanderungs- und Resorptionsgeschwindigkeit annehmen. Andererseits könnte das Eiweiß auch von einem Saccusnahen Sekretionsort oder aus dem Saccus selbst stammen.

Der Einwand, es könne sich bei der autoradiographisch bedingten Schwärzung lediglich um eine Adsorption der markierten Aminosäure an das Eiweiß handeln, läßt sich durch verschiedene Extraktionsverfahren am histologischen Schnitt ausschließen.

Ishii, Silverstein & Balogh (1966) haben radioaktiv markiertes Fremdeiweiß in den Ductus cochlearis des Meerschweinchens eingebracht und dieses Eiweiß nach kurzer Versuchszeit im S. e. nachgewiesen. Nach einer weiteren kurzen Zeit fand sich das Eiweiß zunächst in den frei flottierenden Zellen und dann auch im auskleidenden Saccusepithel der intermediären Portion. Kimura & Schuknecht (1965) erzielten durch Obliteration des S. e. des Meerschweinchens einen erheblichen Hydrops im cochlearen und vestibulären Teil des Innenohres. Durch diese Ergebnisse wird die Theorie eines Longitudinalstromes der Endolymphe zum Saccus hin unterstützt.

4. *Schlußfolgerungen*

Die Ultrastruktur der Saccusepithelien und der intrasacculären Zellen sowie die biochemischen und autoradiographischen Ergebnisse zum Eiweißgehalt bzw. zur Eiweißsynthese im S. e. sind recht gut miteinander in Einklang zu bringen. Danach ließe sich zusammenfassend sagen, daß im S. e. zwei „pha

Acta oto-laryngologica 64: 157–164 (1967)

CONTRALATERAL MASKING AND BÉKÉSY AUDIOMETRY IN NORMAL LISTENERS

B. BLEGVAD
Copenhagen, Denmark

From the Audiological Laboratory, Department of Otolaryngology, University Hospital, Copenhagen, and the State Hearing Center, Copenhagen

The influence of low level contralateral masking on the outcome of Jerger's diagnostic Békésy procedure was studied in normal listeners. In fixed-frequency recordings at 1000 and 4000 cps, masking of the non-test ear was found to separate the tracings for *I* (interrupted) and *C* (continuous tones). At 250 cps no separation occurred and tracings obtained with sweep-frequency technique were always superimposed. As to tracing amplitude, masking caused a statistically significant reduction in both *C* and *I* fixed-frequency tracings at 1000 and 4000 cps. At 250 cps, there was no significant change. The presence of a contralateral noise appears to influence the adaptative mechanisms of the test ear presumably through efferent fibers to the cochlea.

Since our preliminary report was submitted for publication (Blegvad & Terkildsen, 1966), we have repeatedly observed changes in the Békésy audiogram with the introduction of masking noise to the non-test ear. The changes are seen in the sweep-frequency tracings as well as in the fixed frequency recordings. They consist of a separation between the tracings for interrupted and continuous tones and a reduction of tracking amplitude particularly in the latter tracings. In some patients excessive temporary threshold shifts appear in the continuous tone recordings, when masking is applied to the opposite ear. The present investigation was undertaken to examine to what degree contralateral masking would change normal listeners' response to the diagnostic Békésy procedure described by Jerger (1960).

PROCEDURE

The audiometer was a Grason-Stadler E-800. The test signal was delivered through the standard TDH-39 phone mounted in MX 41/AR cushion. Attenuation rate was always "slow" (1.5 *dB* per minute). For masking, the white-noise generator of the audiometer was used, but the second standard headphone was replaced by a Beyer *DT 507* insert type receiver which has been described previously (Blegvad & Terkildsen, 1967). 12

This work was supported by a grant from Statens almindelige Videnskabsfond.

ANDERSEN, H C., 1948 Passage of trypan blue into the endolymphatic system of the labyrinth *Acta Otolaryng* (Stockh) 36 273
ARNVIG, J 1951 Relation of the inner ear to th subarachnoid space and absorption of the labyrinthine fluid *Acta Otolaryng* (Stockh) Suppl 96
BALOGH K und KOBURG, E., 1965: *Der Plexus cochlearis. 36 Tagung Dtsch Ges HNO-Ärzte Hamburg*
v EGMOND, A A J und BRINKMAN W F B 1956 On the function of the saccus endolymphaticus *Acta Otolaryng* (Stockh.) 46 285
EHRICH W E., 1956 Funktion d r Granulocyten und Makrophag n, I : *Hdb Allg Path* VII/1 124
ENGSTRÖM H und HJORTH, S 1950: On the distribution and localization of injected dyes in the labyrinth of th guinea pig *Acta Ol laryng* (Stockh) Suppl. 95 149
GUILD, S R 1927: Circulation of tl e ndolymph *Laryngoscope* 37 649
ISHII, T SILVERSTEIN, H und BALOGH, K 1966 Metabolic ctivities f the endolymphatic sac *An en yme histochemical and aut radiographi study* (Im Druck.)
KIMURA, R S und SCHUKNECHT H F 1965 Membranous hydrops in th inner ear f tl guinea pig aft r obliteration of the end lymphatic sac *Pract Otorhinolaryng* (Basel) 27 343.
KOBURG, E., 1964: Protein m tabolics of the inner ear fluids. *Trans Ame Ol l Soc.,* 19 92
LETTERER, R 1951: *Die Tuberkulose ihre Erk nnung und Behandlung* Stuttgart
LUNDQUIST P-G 1965 The endolymphatic duct and sac in the guinea pig *Acta Otolaryng* (Stockh.) Suppl *01
LUNDQUIST P-G KIMURA R und WERSÄLL, J 1964 a Experiment in end lymph cir cul ti n *Acta Otolaryng (Stockh.) Suppl 188*
— 1964 b: Ultrastructural org nization f the epithelial lining in the endolymphatic duct and ac in the guinea pig *A ta Otolaryng* (Stockh.) 57 65
M\ i n, S H 1948: Further l byrinthi e tudies. I Affecti ns f the human labyri th *Acta Otolaryng* (Stockh.) Suppl. 68
NIKLAS, A und OEHLERT W 1956: Autoradiographisch U tersuchung der Größe des Eiweißstoffwechsels verschied n r Organe Gew be und Z llarten *Beit Path Anat* 116 92
OEHLERT W 1959 *Autoradiographische Untersuch g n bei der xperiment ll n Aspergillose* der Ratte *Acta Histochem* (J na) 6 315
R UCH, S 1964: *Biochemie des Höro gans* Thieme Stuttg rt
SAXEN A 1951 Hist logical tudie of nd lymph secreti n nd resorpti n in the i ner ar *Acta Ol l ryng* (Stockh) 40 23
SCHUKNECHT H F und MCNEILL, R A 1966: Light mi rosc pic bservati ns n the path logy of the ndolymph *Z Laryng Rhin l Otol* 80 1
SEYMOUR, J S 1954: Observati ns n the circul tio in the cochlea *J Laryng* 68 689
SILVERSTEIN, H 1965 Bioch mi l d physi logical studies f the nd lymph tic sac in the cat. *Laryng scope* 76 498

Eingang n am 25 Oktobe 1966

*Priv Do. D E K burg Hal Nasen-Ohrenkl nik d Universität
Sil h rst 5 74 Tubing n
D B K rnbach Hals Nasen-Ohrenklinik d Unive sität
Moor nst 5, 4 Düss ld f
D J Haubrich H ls-N n-Ohrenklin k der Univers tät
G iststr 5/10 34 Götting n Deut hl d*

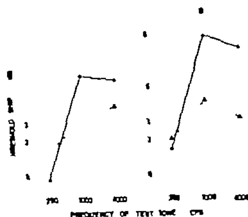


Fig. 2. Mean threshold shifts produced by co tralateral masking as function of test tone frequency. Masking level A, 50 dB SPL B, 70 dB SPL. Tone presentation Δ——Δ, periodically i terrupted; •——• continuous.

given a 15-minute break. These relatively brief intervals were chosen to make the test conditions comparable with those of everyday clinical testing. The duration of the rest periods may be of some importance as following exposure of the non test ear to low level masking noise we have also observed changes in the Békésy tracings. In Fig. 1 the tracings from one of the s bjects are shown.

RESULTS

The tracings were xamined with respect to (1) threshold shifts produced by the masking, (2) separation between *I* and C tracings, and (3) hanges in pen excursion amplitude.

The threshold shifts were determined from the fixed-frequency tracings. In some in tances these tracings showed an improvement of the threshold during the 3-minute period. Quite often—especially in the masked conditions —a hange was seen in the opposite direction, in that the continuous tone threshold slowly drifted downwards during the first minute. After the first minute the thresholds were generally quite stable, and only minor differences were noted between thresholds for the second and third minutes. A the present investigation was to provide a basis for comparison with th changes seen in pathological ears, it was decided to use the second-minute thresholds a the basis for statistical calculations, (for practical reasons, it i not always possible to obtain full 3-minute tracings from patients). A visually interpolated midline between bottoms and tops f the pikes was taken as th threshold. Th mean threshold shift for the different combinations of test tone and masking level are shown in Fig. 2.

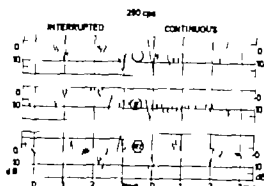


Fig. 1a.

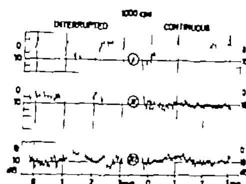


Fig. 1b

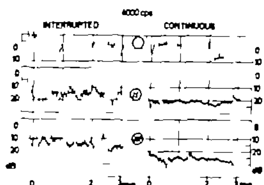


Fig. 1c

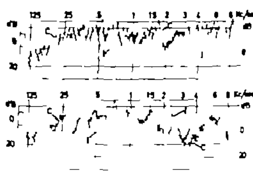


Fig. 1d

FIGS. 1 *a*, *b* and *c*. Fixed frequency tracings for 250, 1000 and 4000 cps respectively obtained with various levels of contralateral masking. Masking level *I* 0 dB; *II* 50 dB; *III* 70 dB. The tracings are from a 22 year old male subject in whom the reduction of tracing amplitude with masking was particularly pronounced.
FIG. 1 *d*. Sweep-frequency tracings for interrupted (*I*) and continuous (*C*) tones obtained in the named subject in quiet (*above*) and with 70 dB contralateral masking (*below*). The audiometer was calibrated to ASA 1951 standard (lower 0-line). The upper zero reference line is an approximation to the ISO 1964 standard.

untrained listeners between the ages of 17 and 23 years were observers. From each subject the following tracings were obtained (1) Sweep-frequency tracings for periodically interrupted tones (*I*) and continuous tones (*C*) without masking and with 70 dB SPL masking of the non test ear (2) Fixed frequency tracings at 250, 1000 and 4000 cps for *I* and *C* with 0, 50 and 70 dB SPL contralateral masking. For these tracings the test tone was started at an inaudible level and 15 seconds were allowed for the *S* to locate the threshold, after which a 3 minute tracing was made. In the analysis of the results the initial 15 second period was disregarded. The different conditions were examined in random order with the exception that the *C* tracing was always obtained immediately after the corresponding *I* tracing. A maximum level of 70 dB for the masking was chosen to reduce the risk of threshold shifts produced by crossmasking. Minimum rest periods between fixed frequency recordings were 45 seconds. After the recording of sweep-frequency tracings with 70 dB masking the subject was

The reduction of amplitude in fixed frequency tracings at 1000 and 4000 cps, combined with the separation between the mid-points of the tracings, often made corresponding C and *I* tracings completely separate when compared by means of superposition of the charts and visual inspection. In the sweep-frequency tracings, only a few subjects showed any appreciable change in the size of the threshold excursions, primarily a reduction in the C curve at higher frequencies, and in all subjects the *C* and *I* tracings interweaved even in the high-frequency range.

DISCUSSION

The results demonstrate a definite influence of contralateral masking on the outcome of Békésy audiometry in normal listeners. Important changes were the separation between the *C* and *I* fixed-frequency tracings seen at 1000 and 4000 cps and the reduction of tracing amplitude in these recordings. Although the tracings were superimposed or overlapping in some subjects, in other subjects even the lowest masking level (50 dB) was sufficient to cause a conspicuous separation. However, even in subjects with a clear separation of fixed-frequency tracings, the sweep-frequency recordings were still superimposed. As Jerger's classification of Békésy audiograms is based on the joint outcome of fixed frequency and sweep-frequency tracings, the masking employed did not change the normal listener audiogrammes to a typical type II. With regard to the reduction of tracing amplitude the mean changes were greater for *C* than for *I* tracings, but statistical analysis proved that the alterations were significant for both. The narrowing of the continuous tone tracings took place gradually and continued after the threshold had become stable (cf. Figs. 1 *b* and *c*).

Sillinger & Elliott (1960) investigated Békésy fixed-frequency tracings in normal listeners after ipsilateral exposure to fatiguing pure tones. They found greater temporary threshold shifts for continuous than for interrupted tones, and a reduction of tracing amplitude which was more pronounced in the continuous tone tracings. According to these investigators, "The difference in threshold location between pulsing and continuous tones can be ascribed to the increase in information transmission owing to the ability to make a number of noise and signal + noise comparisons when the tone is pulsed. In other words, when the tone is periodically interrupted it is easier for the subject to decide whether tone is present or absent and the threshold can be traced at a lower SPL. Further: "The difference in size of Békésy audiometer pen excursions between continuous and pulse tones, when found, and the additional decrease in size during auditory fatigue is a function of the ability to perceive and react to the rate of loudness growth directly. When the tone is interrupted—chopped up so to speak—the perception of loudness growth is rendered more difficult. Therefore in conditions accompanied by an abnormally rapid loud

TABLE 1 *Separation (dB) between I and C tracings in quiet and with contralateral masking*

Positive entries signify that *I* tracings were traced at lower sound pressure levels than *C* tracings

Masking level (dB)	250 cps			1000 cps			4000 cps		
	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range
0	1.3	1.3	−4.5 to 5.0	1.3	1.0	−2.5 to 6.0	4.1	4.5	−0.5 to [illegible]
50	−0.4	0	−5.0 to 5.0	3.6	2.5	−2.0 to 13.0	5.6	5.3	1.0 to 11.5
0	0.5	0.3	−4.0 to 5.0	4.4	3.3	−1.5 to 1[illegible].0	7.2	6.0	0 to 20.0

At 1000 and 4000 cps the mean shifts were greater for continuous tone tracings than for periodically interrupted. In some subjects the shift of the *C* threshold was quite pronounced as compared to the change in *I* threshold. In other subjects the two thresholds were shifted equally. Furthermore in the unmasked condition many subjects had better thresholds for interrupted tones (which is in agreement with the reports of Rosenblith & Miller 1949 and Silbiger & Elliott 1960). For these reasons the results were analysed with respect to the separation between *I* and *C* tracings occurring in the individual *S* (Table 1). Separation it should be noted, indicates the difference between *mid points* of tracings. A positive entry signifies that the threshold for interrupted tones is the more acute. In order to supplement the figures of the table it can be mentioned that in one third of the subjects the separation became > *7* dB at 1000 cps and > 10 dB at 4000 cps when 70 dB masking was applied.

The tracing amplitudes as determined from the fixed frequency tracings, are given in Table 2. In each tracing the mean amplitude was calculated for the first, second and third minute separately by counting the number of penswings per minute and converting to dB in the usual way (pen excursion amplitude (dB) = attenuation rate (dB per min)/number of penswings (per min)). At 1000 and 4000 cps, the mean amplitude of the *C* tracings gradually decreased from the first to the third minute for which reason the amplitudes are stated for each of the 3 minutes.[1] In the rest of the tracings no systematic variation by time was demonstrable and only the mean value for the entire 3 minute period is given. The tracing amplitudes of the individual *S* in quiet and with masking were compared and by means of the *t* test we controlled whether mean intercondition variations were statistically significant from 0. At 250 cps no significant change occurred but at 1000 and 4000 cps masking caused a significant reduction of the tracing width in both *C* and *I* tracings.

[1] At 1000 cps, this gradual narrowing of the tracings was most pronounced in the masked conditions, and with 70 dB masking the mean difference between values for the second and the third minute was found to be significant ($P < 5$ %)

quiet and with contralateral masking

– to be significant (and for $P < 5$ and < 1 respectively).

4000 cps

Continuous tone					Interrupted tone		Continuous tone				
Amplitude				Change	Amplitude	Change	Amplitud				Change
1st min	2nd min	3rd min	Average				1st min	2nd min	3rd min	Average	
40	7.37	7.33	7.37		7.39		6.10	5.85	5.42	5.77	
6.21	5.77	6.43	5.80	−1.57	6.52	−0.87	4.59	4.44	4.46	4.50	−1.28
5.48	5.29	4.97	5.27	−2.09*	5.67	−1.52*	4.20	3.74	3.71	3.67	−1.91

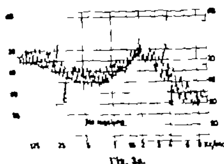


Fig. 3a.

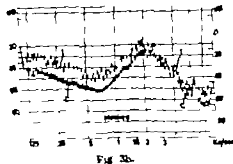


Fig. 3b.



Fig. 3c

Fig. 3 S erp-frequency tracings in 56-yea old mal obtained one week fter a acute ttack of Ménière's Disease. The test tones were d li ered by I sert-type recei er no masking wa pplied t the non-test ea

Fig. 3 b S erp-frequency tracings i the same patient hen 70 dB masking was delivered t the contralateral ea by insert recei er during the exami tion.

Fig. 3 The correspondi g fixed-frequency tracings (obtained the f llowing d y) Test one frequency a, 250 cps b 1000 cps; 4000 cps.

TABLE 2 *Mean tracing amplitude (dB)* i
Asterisks indicate that the changes produced by masking were fou

Masking level (dB)	250 cps Interrupted tone Amplitude	Change	Continuous tone Amplitude	Change	1000 cps Interrupted tone Amplitude	Change
0	7 65		8.50		8.09	
50	8 25	0 60	8.63	0.12	7 07	[illegible]
70	7.57	−0.08	7 60	−0.85	6.41	[illegible]

ness growth the reduction will be less pronounced for *I* than for *C* tracings. These fundamental viewpoints might well pertain to the changes seen in Békésy tracings during contralateral masking

Dirks & Norris (1966) compared the effect of various ipsilateral and contralateral maskers on Békésy fixed frequency tracings in normal listeners Their study included the use of continuous and interrupted wide-band and narrow band noise as well as of pure tone maskers of various frequencies. The results quoted below are for the continuous wide-band white noise masking Contralateral masking was found to shift the continuous tone thresholds more than the interrupted tone thresholds at 1000 and 4000 cps which is in agreement with our findings. The separation between tracings occurring in the individual *S* is not stated and sweep-frequency examinations were not carried out As to tracing amplitude during contralateral masking they found a considerable reduction in the *C* tracings at 4000 cps at 1000 cps there was only a small reduction and in the *I* tracing no seizable change occurred at either frequency Their tracings were for one minute only and in the experiment in which the amplitude was measured the masking level was only 40 dB SPL During ipsilateral masking, *I* and *C* tracings were shifted equally and little change in tracing amplitude was observed This corroborates the findings of Small & Minifie (1961) who found no significant change in amplitude during homolateral masking (masking level 60–110 dB)

The effect of contralateral masking in normal listeners appears to differ at least in degree from what may be observed in patients with unilateral perceptive hearing loss In none of the normal listeners did we see separation of sweep frequency tracings neither did we see the separation of fixed frequency tracings at 250 cps, that may be seen in patients (Fig 3) Finally we never saw the precipitous threshold shift seen in some patients (Fig 4) The maximum separation between *I* and *C* tracings in any normal listener at any frequency was 20 dB

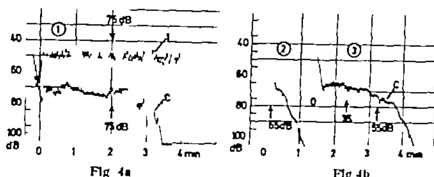
According to Harbert & Young (1962 1964 and 1966) the reduction of

REFERENCES

DUNOVAN, B., and TERKILDSEN, K., 1966 Békésy audiometry SISI-test and contralateral masking Preliminary report. Act Otolaryng (Stockh.) 62 453.
— 1967 Contralateral masking and the SISI-test in normal listeners. Act Otolaryng (Stockh.) 63, 547
DIRKS, D D and NORRIS, J D 1966 Shifts in auditory threshold produced by ipsilateral and contralateral maskers at low-intensity levels. J Acoust Soc. Amer 40 12.
HARBERT F and YOUNG, I M., 1962: Clinical application of hearing tests. Arch. Otolaryng (Chic) 76 85.
— 1964 Audiologic findings in Ménière' syndrome. Act Otolaryng (Stockh.) 57 148.
— 1966 Amplitude of Békésy tracing with different attenuation rates. J Acoust Soc. Amer 39 914
JERGER, J 1960 Békésy audiometry in analysis of auditory disorders. J Speech Hearing Res., 3, 275
ROSENBLITH, W A and MILLER, G. A., 1949: The threshold of hearing for continuous and interrupted tones. J Acoust. Soc Amer 21 467
SHANNON, H. R and ELLIOTT D N 1966 Auditory threshold location and uncertainty function of tone parameters and fatigue J Acoust Soc Amer 39 117
SMALL, A. M Jr., and MINIFIE, F D 1961 Intensity differential sensitivity at masked threshold. J Speech Hearing Res., 4 164
SUZUKI, T F ... T and ..., T 1962 A consideration on reduced amplitude in Békésy audiograms. J p. J Otol (Toky) 65, 731

60 Vingaards allé,
Hellerup Denmark

Received January 9 1967



FIGS. 4 *a* and *b* Consecutive fixed frequency examinations in a 60-year old femal with a right, flat type sen ory neural lo s of unkn wn origin. The test tone (4000 cps) and the ma king noise were gi n through insert. Pure tone audiometry of the left ear showed a pron unced high frequency loss, and at 4000 cps hearing was almost equally impaired n the two ides. (1) Tra ing obtained f r 2 minutes in quiet after which 75 dB contralateral ma king was introduced (2) The maske was introduced immediately bef re the test t ne (3) Step-wise introduction of the ma ki g

amplitude and the slight separation between tracings found in certain pathological ears are caused by abnormal rapid adaptation a wide separation is thought to be due to slow adaptation Also Suzuki *et al* (1962) ascribed reduction of amplitude to adaptation Our investigations demonstrate that in normal and pathological ears alike, the tracing amplitude as well as the degree of separation can be influenced by even low level contralateral masking They also demonstrate that the alterations in the continuous tone tracings take place quite gradually which seems to imply that a humoral transmitter or some metabolic change may be involved Pure psycho-acoustic phenomena can hardly be responsible since the presence of an ipsilateral masker has an entirely different effect. The Békésy tracings obtained in normal listeners during contralateral masking resemble those obtained in patients with typical end-organ lesions such as noise-induced hearing loss *It would be reasonable to assume that masking* of the non test ear exerts an influence on the test ear via efferent fibers and that the effect is closely related to adaptative phenomena

ZUSAMMENFASSUNG

Der Einfluss kontralateraler Maskierung von niedriger Intensität auf das Ergebnis des Jergerschen diagnostischen Békésy-Verfahrens wurde bei Normalhörenden studiert Bei frequenzkonstanten Aufzeichnungen mit 1000 und 4000 Hz hat die Maskierung des nicht gepruften Ohres eine Trennung zwischen den Kurven *C* (stetige Töne) und *I* (pulsierende Töne) verursacht Bei 250 Hz hat keine Trennung stattgefunden und die Kurven welche mit gleichmässig steigender Frequenz aufgezeichnet wurden waren stets überlagert In bezug auf die Kurvenausschläge hat die Maskierung bei sowohl *C* als auch *I* bei frequenzkonstanten Kurven mit 1000 und 4000 Hz eine statistisch signifikante Reduktion verursacht Bei 250 Hz kamen keine signifikanten Änderungen vor Die Anwesenheit kontralateralen Geräusches scheint die adaptiven Mechanismen des geprüften Ohres zu beeinflussen vermutlich durch efferente Fasern zur Cochlea

In view of the conflicting reports of the literature we have undertaken to ascertain which of the two theories has a validity from the clinical and experimental observations.

Attention was directed to the following points

(1) Does a precise *localization for* the cortical area which influences the vestibular apparatus and produce DP of induced nystagmus truely exist or not?

(2) *Is the occurrence of* DP of induced nystagmus affected significantly by the difference of test procedures, i.e. test with optic fixation maintained and test with the eyes closed?

(3) *Is the vestibular* test of no value in the localization of cerebral lesions, as concluded by Hakas & Kornhuber?

METHOD AND RESULTS

1 *Clinical observations*

The clinical observations were performed on 43 patients with a tumor occupying, in the main, the cortical area. In all cases the diagnosis was confirmed by operation and histological examination, and in some cases, by autopsy Complete data for all 43 subjects relating to DP obtained from the differential caloric test are summarized in Table 1

Furthermore data relating to spontaneous, positional nystagmus and estibular reaction f r 15 patients with a tumour mainly limited to the unilateral temporal lobe are given in Table 2.

Of 15 subjects 9 cases and DP of vestibular nystagmus 3 cases were toward the lesion and 6 cases were to the side opposite the lesion

Of the total of 43 cases of unilateral cerebral lesion, 20 cases had DP and nly 0 cases were toward the lesion

In 2 cases (Nos. 13 and 15 in Table 2) cal rization was performed with two different conditions, according *to Hallpike s theory i.e firstly with* actly optic fix tion and secondly with the eyes closed, and the change f the % of DP w s compared The results are shown in Table 3

In N 13, *no el ange of the % of DP* was observed and in No *15 little* decline of the % of DP was observed in the condition of eye-opening. Those result re diff rent from Hallpike s.

TABLE 1 *Vestibular DP f 43 patients with cerebral tumors occupying cortical area*

Localization	Number	Number of subject showed DP	DP to the affected side	DP to the unaffected side
Frontal lobe	11	4	2	2
Parietal lobe	14	6	4	2
Temporal lobe	15	9	3	6
Occipital lobe	3	1	0	1

Acta oto-laryngologica 64 186–173 (1967)

DIAGNOSTIC VALUE OF DP PHENOMENON IN THE NYSTAGMIC REACTION

Y Koike, K Mizukoshi A Wada A Haba,
T Takahashi M Takashima and H Ino
Niigata Japan

From the Department of Otolaryngology (Head Prof H Ino)
Niigata University School of Medicine

(1) DP phenomenon shows no direct relationship to a lesion in a particular part of a particular hemisphere as previously reported by Hakas & Kornhuber (2) The region of responsibility for the development of DP will be located in some parts level with the subcortical area or the brain stem. (3) By the use of focused ultra-sound irradiation pronounced DP to the side of the lesion could be observed from the lesions of the lateral portion of ponto-mesencephalic reticular formation in the rabbit.

INTRODUCTION

Directional preponderance was previously defined by the German term "Nystagmusbereitschaft Fitzgerald & Hallpike (1942) from systematic studies using their so-called differential caloric test substituted "Directional Preponderance for "Nystagmusbereitschaft" Carmichael Dix & Hallpike (1954) found that brain tumors located in the posterior portion of the temporal lobe cause a directional preponderance of caloric induced vestibular nystagmus to the side of the lesion but did not find DP with tumors of the frontal parietal or occipital lobe

Hakas & Kornhuber (1959) published their studies of DP in 201 cases of localized cerebral lesions. They found only 31% of the cases had DP of vestibular nystagmus. Of these 67% were to the side opposite from that of the lesion, and 33% toward the lesion Their conclusions, very different from those of Hallpike (see Fitzgerald & Hallpike, 1942 Carmichael Dix & Hallpike, 1954) were that DP showed no direct relationship to a lesion in a particular part of a particular hemisphere

In answer to Hakas & Kornhubers paper Carmichael et al (1961) stated that this striking disparity between the two results can be attributed to the difference of techniques of physiological conditions of the test procedure employed, namely the former tests were carried out with active optic fixation and the latter with the eyes closed The abolition or reversal of DP when tests were carried out in the manner of Hakas and Kornhuber with the eyes closed might be due to conjugate deviation of the eyes which may occur under these conditions

TABLE 3. *Change of DP (%) caused by different conditions in patients with temporal lobe tumors (Caloric nystagmus)*

Number of case	Localization of tumors	Condition	Duration (sec.)	Direction of DP	DP (%)
No. 13	Post. inf. part of rt. temporal lobe	Eyes closed	387	T the unaf fected side	23
		Eyes opened with optic fixation maintained	365	T the unaf fected side	12
No. 15	Post. part of rt. temporal lobe	Eyes losed	411	T the unaf fect d side	28
		Eyes opened with optic fixation maintained	319	T the unaf fected side	22

calized lesions in the brain of rabbits. The apparatus employed, generating highly intensive focused ultrasound, consisted of the power supply and the transducer The procedure of irradiation was the same as previously reported by Sasaki (1965) who is a member of our clinic. Therefore the details of the procedure employed have been omitted from this paper

After the removal of the t mporal bone the continuous focal lesions were produced on 10 points of the temporal lobe in the rabbit at a distance of 3 mm in two rows. After operation, vestibular nystagmus was observed for a long period of tim

Calorization was performed with 20 cc of 10 C and 52 C. The changing pattern f the % of vestibular DP obtained from Hallpike s method one month after irradiation i shown in Fig. 1

Although a r latively large dispersion of the % of DP was observed, ultimately the direction of DP might be considered to be toward the af fected side but it remained only within a change of 10% i.e within the normal limit Therefore one cannot insi t that this slight change of the % f DP ha a significance in deciding the direction of DP And yet, it is worth mentioning that the change of the of DP was not influenced by the difference of the test conditions, throughout the course of our experiments.

The hi tological finding at 31 days after irradiation was that necrosis nd softening of cortical ti ue wa mainly present in the left temporal lobe and partially present in the left parietal and occipital lobe.

In some part of the whit matter swelling nd di ruption of cell and fibers wa observed, but no other accessory destruction below the white matter was seen.

TABLE 2 *Vestibular DP of patients with temporal lobe tumors (15 cases)*

Number of cases	Localization of tumors	Histological findings	Spontaneous nystagmus	Positional nystagmus	Vestibular reaction
1	Ant halves of *rt. temporal lobe*	Meningioma	∤	∤	DP to the right (unaffected side)
2	Post part of lt. temporal lobe	Spongioblastoma *polare*	←	∤	∤
3	Ant part of rt. temporal lobe	Meningioma	∤	∤	DP to the left (unaffected side)
4	Ant part of rt temporal lobe	Meningioma	∤	∤	DP to the right (unaffected side)
5	Ant. part of rt temporal lobe	Meningioma	∤	∤	DP to the right (unaffected side)
6	*Post. part of* lt. temporal lobe	*Astroblastoma*	←	→ ←	*DP to the right* (unaffected side)
7	Ant middle part of rt temporal lobe	Astrocytoma	∤	∤	∤
8	Middle part of rt. temporal lobe	Abscess	∤	∤	∤
9	Ant part of lt temporal lobe	Meningioma	←	∤	DP to the right (unaffected side)
10	Rt. fronto-parieto-*temporal lobe*	Fibrillary *Astrocytoma*	∤	∤	∤
11	Middl part of lt. temporal lobe	Glioma	∤	∤	∤
12	Post part of lt temporal lobe	Astrocytoma	←	←	DP to the right (unaffected side)
13	Post inf part of rt. temporal lobe	Haemangioma	→	∤	DP to the left (unaffected side)
14	*Rt parieto-temporal* lobe	*Subcortical* Ependymoma	∤	∤	∤
15	Post. part of lt. temporal lobe	Glioma	←	←	DP to the right (unaffected side)

To clear up the change of the % of DP caused by the difference of test conditions, we *indiscriminately* selected *18* patients who showed *DP to one* side and reinvestigated on the change of DP phenomenon The results are shown in *Tables 4 a* and *4 b*

The reversal of DP was observed only in one case (No 7) In 4 cases the increase or decrease of the % of DP was manifested by eye-opening, and in the other cases no change of the % of DP was observed Moreover no DP phenomenon of *optokinetic* nystagmus was observed in No 7 which showed the reversal of vestibular DP

2 *Experimental observations by means of ultrasonic irradiation*

Hitherto, the method *of absorption or electrocoagulation* has usually been resorted to in order to make lesions in the brain In the present series of experiments, *focused ultrasonic irridation* was *employed* to make lo-

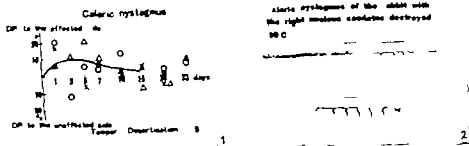


Fig. 1. The ch ging pattern of the % f cutfbala DP after irradiation.
Fig. 2 Caloric ys t gmus of the rabbit with the right ucleus ca datus destroyed. The secondary phase of ys tagmus was manifested.

In clinical practice it is generally appreciated that, of those characteristics—f r example duration, number of beats, maximum eye-speed in the l w phase—duration is the most reliable factor for estimation of DP phenomenon and many investigators have proposed a limit of 20% for duration in the determination of DP Judging from this limit, if the reversal of DP had occurred because of the difference of test procedures as Hallpike tated, it mean that a change of more than 40% in total duration would appear If this were true we could safely say that the caloric test is not uitable f r estimation of vestibular function But, in reality any significant difference could not be observed.

Therefore our conclusions are the same as Hakas & Kornhubers That is, DP phenomenon show no direct relationship to a lesion in a particular part f a particular hemi phere The region of responsibility for the devel pment of DP will be located in some parts level with the subcortical area or the brai tem, but not located in a hemisphere

Accordingly to clear up this problem, we are now making localized le i m in some parts—for example nucleus caudatus, thalamus, cerebellum r ticular f rmation and medial longitudinal fasiculus, and so on, which

Table Normal limit of DP in caloric nystagmus

M. E. S. Maxim m eye-speed.

	Duration (sec)		Number of beats	M.E.S. (°/sec)
Hallpik (1932) / Thomsen (1953)	40			
Jongkees (1953)		20		
Pfalt (1957)	100	20	210	10
Stahle (1958)	60	20	175	18
Orlandi (1962)	100	15	130	
M La (1962)	40		40	8

TABLE 4 *a Change of DP (%) by different conditions*

No of cases	Direction of DP		DP (%)	
	Eyes closed	Eyes opened	Eyes closed	Eyes opened
1	DP (–)	←	3.2	10.6
2	←	←	19.2	20.3
3	DP (–)	←	37 0	28.5
4	→	→	14 0	22.0
5	→	DP (–)	19 1	4.1
6	→	DP (–)	16.0	8.0
7	→	←	19.2	18.5
8	←	←	14.5	23.0
9	→	→	21.5	19 0
10	→	DP (–)	16.0	8 0
11	→	→	15.0	16.0
12	DP (–)	←	11 0	23 0
13	←	←	23.0	21 0
14	→	→	26.0	27.0
15	←	←	13.5	14.5
16	←	←	1 .5	9.5
17	→	→	23 0	22.0
18	→	→	15 6	16 3

When these results were compared with those of temporal decortication performed by surgical procedure the change of the % of DP in vestibular nystagmus was relatively larger than the results of ultrasonic irradiation. But this increase of the % of DP disappeared within 10 or 15 days after irradiation And the % of vestibular DP was not changed by the difference of the test conditions, i e with eyes closed or eyes opened during the course of the experiment

COMMENTS

As for the statistical normal limit of DP obtained from the differential caloric test of Fitzgerald & Hallpike (1942) many reports (Fitzgerald *et al* 1942 Thomsen 1953 Pfaltz, 1957 Stahle 1958 Hamersma, 1957 Otani, 1962 McLay 1962) have been accumulated up to the present day as shown in Table 5

TABLE 4 *b Change of DP (%) caused by different conditions (18 cases)*

	Number
Decline of DP (%) with eyes opened	4
Increase of DP (%) with eyes opened	4
Alteration of the direction of DP with eyes opened	1
No-change of DP with eyes opened or closed	9
Total	18

Destruction below the level of the trochlear nucleus and partial central grey substance total reticular formation, partial rostral pontine reticular nuclei of raphe and total predorsal fasticulus elicited a unilateral optokinetic inversion. This fact was already reported by Sasaki (1965)

The lesions of the lateral portion of ponto-mesencephalic reticular formation caused marked DP towards the side of the lesion.

We have considered that the lesion is not extended to the vestibular nuclei and its neighbouring fibers because there was no occurrence of spontaneous or positional nystagmus. But any decisive conclusion concerning the responsibility of DP phenomenon could not be obtained because of extensive destruction as shown in Fig 5 This problem requires further observation and discussion in the future.

ZUSAMMENFASSUNG

1) Die Nystagmusbereitschaft (Richtungsüberwiegen des Nystagmus) zeigt überhaupt kein Korrelation zu bestimmten Lokalisationen der Grosshirnläsionen, wie von Hakas u. Kornhuber (1959) schon angenommen wurde 2) Die subkortikale Region oder der Hirnstamm hat eine besonders wichtige Rolle bei Hervorrufung der Nystagmusbereitschaft 3) Die Läsionen der lateralen Teil der pontomesencephalen Formatio reticularis, mittels des hoch intensiven fokalen Ultraschalls erzeugt rufen die auffallend Nystagmusbereitschaft zur Herdseite hervor

REFERENCES

Carmichael, E. A. Dix, M. R., and Hallpike, C. S., 1954 Lesions of the cerebral hemispheres and their effect upon optokinetic and caloric nystagmus. Brain, 77 345

1961 Some further observations upon the effect of unilateral cerebral lesions on caloric and rotational nystagmus. Brain 84, 571

Fitzgerald, G. and Hallpike, C. S., 1942 Studies in human vestibular function. 1 Observation on the directional preponderance (Nystagmusbereitschaft) of caloric nystagmus resulting from cerebral lesions Brain 65, 115.

Hakas, P. and Kornhuber, H., 1959 Der vestibulare Nystagmus bei Grosshirnläsionen des Menschen. Arch Psychiat 200 19

Hennebert, ... H., 1957 The Caloric Test. ... Amsterdam

McLa... K. 1962 ... assessment of method of vestibular testing ... clinical practice J Laryng 76, 34

... T. 1962 Which is the best ... estimate the vestibular function? J ... J Otolaryng Tokyo 65, 424

Pfaltz, C. R., 1957 Die normale calorische Labyrinthreaktion Arch. Ohr Nas. K ... 171, 2, 131

S... T. 1965 On the ... of nystagmus caused by Ultrasound-produced focal lesions in the brain stem. Acta Otolaryng (Stockh.) 60 354

S... J. 1953 Electronystagmography in the caloric and rotatory tests. Acta Otolaryng Stockh Suppl. 13...

Torok ... 1953 The caloric test ... Hallpike ... in ... normal material Acta Otolaryng (Stockh. Suppl. 109 189

Dept of Otolaryngology
Niigata University School of Medicine
Niigata, Japan

Received September 5, 1966





Fig 3 Histological finding of a lesion f the nucleu caudatus.
A Corpu call sum B Nucl caudatus. C: Capsula interna. D Putamen. E Capsula xtern F Part of lesion

Fig 4 Hi tological finding of a lesion of the thalamus opticus.
A: Nucl v ntrali of thalamus. B Lamina medullari ventralis. C Subst tia retic ti al mi D Nucl medialis f thalamus. F Nucl lat rali of thalamus. F: Lamina medullaris medialis G: Nucl ce tralis thalami H Parts of the lesion

are of vital importance in the evocation of normal nystagmic reaction by the use of ultrasonic irradiation

However the data are not complete up to the present day To indicate some results of our experiments, we can say that the focal lesion of the thalamus or nucleus caudatus caused no DP phenomenon, but the secondary phase of vestibular nystagmus became manifest



Fig 5 Histologi l find ng of th bbit whi h h wed DP ma ked ft r local ed de t u ti n of the b in t m
A Colliculus superior B S bst grisea ce trali C Colliculu inf ri D F L P E F mati reticulari F Lem scu medialis. G: Pyramis. H Raph I P rt of th le i n

Destruction below the level of the trochlear nucleus and partial central grey substance total reticular formation, partial rostral pontine reticular nuclei of raphe and total predorsal fasciculus elicited a unilateral optokinetic inversion. This fact was already reported by Sasaki (1965)

The lesions of the lateral portion of ponto-mesencephalic reticular formation caused marked DP towards the side of the lesion

We have considered that the lesion is not extended to the vestibular nuclei and its neighbouring fibers because there was no occurrence of spontaneous or positional nystagmus. But any decisive conclusion concerning the responsibility of DP phenomenon could not be obtained because of extensive destruction, as shown in Fig. 5 This problem requires further observation and discussion in the future.

ZUSAMMENFASSUNG

1) Die Nystagmusbereitschaft (Richtungsüberwiegen des Nystagmus) zeigt über haupt keine Korrelation zu bestimmt n Lokalisatio en der Grosshirnläsionen, wie on Hakas u. K rnhuber (19[illegible]) schon ngen mmen wurde 2) Die subkortikale Region oder der Hirnstamm hat eine besonders wichtige Roll bei Hervorrufung der Nystagmusbereitschaft. 3) Die Läsionen der lateralen T il der pontomedullären Formatio reticularis, mittel des hoch intensiven f kalen Ultraschalls erzeugt, rufen die auff llend Nystagmusbereitschaft zur Herdseite hervor

REFERENCES

[illegible], C A., D[illegible], M. R., and H[illegible], C. S., 1954 Lesions f the cerebral hemispheres nd thei effect pon optokinetic nd caloric nystagmus. *Brain*, 77 345.

1961 Some futher observ tion po the effect of unilateral cerebral lesions on caloric nd rotational ystagmus. *Brain*, 84 [illegible]

F[illegible], C and H[illegible], C. S., 1942 Studies in huma estibula function 1 Observations on the directional preponderance, Nystagm sbereitschaft of aloric ystagmus resulting from cerebral lesions. *Brai* 65, 115.

H[illegible], P nd Korn [illegible], H 1939 Der estibulare Nystagmus bei Grosshirnlä ionen des M nschen. *Arch Psychiat* [illegible] 19.

H[illegible], H 1937 The Caloric Test. *Diss.* Amsterdam.

M[illegible] K 1962 A assessment of methods of estibular testing i cli ical practice *J Laryng* 76, 31

O[illegible] T 1962 Which is the best lue t estimat the estibular f nction? *J p J Hol ryng Tokyo*, 65, 624

P[illegible], C R., 1937 Die normale calorische Labyri threaktion *Arch Oh Nas. K hl k pfheilk* 172, 131

S[illegible], T 196[illegible] On the ha ges f ny t gm caused by ltrasound-produced focal lesion in the brain-stem. *Act Otolaryng* (Stockh.) 60 394

S[illegible], J 19[illegible] *Electronyst gmograph* in the caloric nd rotatory tests. *Act Otolaryng* Stockh Suppl. 137

T[illegible] K A 1933 The caloric test m. Hallpike f al l normal material. *Act Otolaryng* Stockh.) Suppl *109* 189

Dept f Otolaryngology
N igat U versity School of Medici
N igata, J pa

Received Septembe 5 1966

Acta oto-laryngologica 64 174-178 (1967)

THE SCHREINER PHENOMENON IN THE LIGHT OF NEW INVESTIGATIONS

EMILIA KROCHMALSKA Z MNICH and T LUPINSKI
Bialystok Poland

From the Clinic of Otolaryngology (Head Prof W Hassmann) and the Department of Medical Physics (Head E Trembaczowski) Medical Academy Bialystok

The authors have confirmed the observation by Schreiner that the isotope from the perilymph of the first ear passes to the perilymph of the second ear and its activity is greater than the activity of cerebrospinal fluid in the cisterna magna and serum. At the same time they found that the isotope migrates by way of the subarachnoid spaces and cisterns of the base of the cranium The low activity of the cerebrospinal fluid in the cisterna magna was probably due to the fact that the fluid in the cisterna magna came chiefly from the IVth ventricle which is by passed by the isotope introduced into the perilymph of the first ear on its way through the cisterns of the base of the cranium The low activity of the serum is connected with the small surface of exchange

In 1961 Schreiner found that after introducing radioactive phosphorus into the perilymph of one ear the activity of the perilymph of the opposite ear one hour later was considerably higher than that of the serum and cerebrospinal fluid obtained by suboccipital puncture According to the author this result "justifies a search for a direct communication between the two cochleae or along the nerve sheats" Rauch (1964) supplemented Schreiner s observations with his own which partially confirmed those of Schreiner regarding the observed effect as a phenomenon

The aim of our investigations was to repeat Schreiner s experiment and to trace the paths by which radioactive phosphorus migrates to the perilymph of the opposite ear

METHODS

Six cats were used for the experiments. Na $Hp^{32}O_4$ was administered to each of the cats through the round window both of the windows having been sealed previously with rubber solution In order to exclude the possibility of the isotope entering the cerebrospinal fluid of the sub-

arachnoid cisterns of the base of the cranium from the blood Identical experiments were carried out on two dead animals After aspiration of the perilymph, half a drop of the isotope that is, a smaller amount than that administered to the other animals, was administered to animal No. 1 The remaining animals received one drop of the isotope, the method employed in its administration being similar to that of Schreiner that is, without aspiration of the perilymph and pressure being applied when introducing the isotope One hour later blood was taken through a cardiac puncture and immediately after death the cerebrospinal fluid was obtained by means of a suboccipital puncture and perilymph was withdrawn from the opposite ear Then after removal of the calva, the cerebrum was lifted and cerebrospinal fluid was btained from the subarachnoid spaces of the base of the cranium The fluids thus obtained were weighed on a chemical balance and their activity was determined by means of a Geiger-Müller counter with electronic scaler Type LL-1 and calculated per 10 mg of the investigated fluid

RESULTS

The table on page 170 shows the behavior of the activity of the various fluids.

The authors found similarly to Schreiner that when the isotope was introduced into the perilymph of one ear the activity of the perilymph in the opposite ear was higher than the activity of the blood and the cerebrospinal fluid obtained from the cisterna magna by means of suboccipital puncture

The activity of the perilymph in the opposite ear appears to be dependent on the amount of isotope applied, and on the pressure under which it is introduced into the perilymphatic space In the case of animal No. 1 which received half a drop of the isotope after aspiration of the perilymph n activity whatever was noted in the perilymph of the opposite ear On the other hand, in animals Nos. 5 and 6 which received a double quantity f the isotope applied under pressure, marked activity was noted in the perilymph of the opposite ear

From the observations the behavior of the activity in the subarachnoid paces and the cistern of the base of the cranium, it may be concluded that the path of the isotope from the perilymph of the first ear leads fr m the subarachnoid spaces near the site where the cochlear aqueduct enters the cisterna anguli pontocerebellaris, the cisterna ponto-cerebellaris and the cisterna medullae oblongatae which forms part of the ci terna pontomedullaris, nd from there the isotope passes to the opposite ide of the base f the cranium t the cisterna ponto-cerebellaris nd cisterna anguli ponto-cerebellaris, from where it reaches the subarachnoid spaces near the opening f the cochlear aqueduct and enters the perilymph of the opposit ear

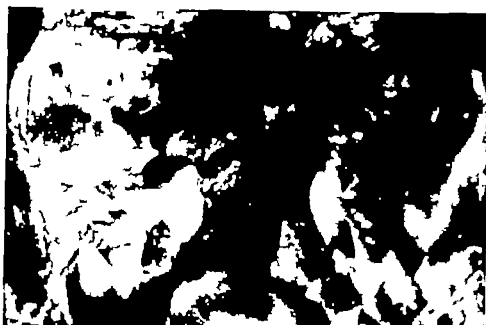


FIG. 1 Phot graph of the posterior and medial cranial fossa of a cat.

TABLE 1

Animal n	Activity no. f impulses (10 mg/l min)				
	C.S.F from the region of cochlear aque duct opening—first ear	C.S.F from c ponto-cere-bellaris—first ear	C.S.F from c. ponto-medul laris	C.S.F from c. I terpedun cularis	C.S.F from c basilis near n.V
I	—	4850	112	—	—
II	315	460	490	—	—
III	810	700	477	192	122
IV	—	1410	2620	184	290
V (dead)	750	3150	1120	—	480
VI (dead)	—	4200	3700	28 0	164
	C S F from c. po to-cere-bellaris—opposit ear	C.S.F from the regi n f cochlear aque-duct openi g—pposite ear	Perilymph from opposit ar	C.S.F from c. cerebello-medullari	Serum
I	20	—	0	1	25
II	80	75	28	1	26
III	142	183	20	12	18
IV	2100	—	56	6	40
V (dead)	10 0	580	240	40	—
VI (dead)	1180	550	140	9 0*	—

Th isotope was dmini tered twice d greater pressure thus ca l g the isot pe t enter c cerebello-medullaris and c. interpeduncularis.

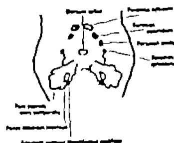


Fig. 2. Diagram of the posterior and medial cranial fossa of cat with dotted line marking the assumed path of the isotope.

Pneumography performed for diagnostic purposes in persons in whom changes in the region of the anguli ponto-cerebellaris were suspected shows that isotope migrates by this path (Kautzky & Zölch, 1953).

The absence of activity or only slight activity in the cerebrospinal fluid obtained by means of a suboccipital puncture could be due to the fact that the cerebro-spinal fluid in the cisterna magna came mainly from the fourth ventricle (Clara, 1953) which the isotope introduced into the first ear by passes on its way through the subarachnoid cisterns of the base of the cranium. The main current of the cerebrospinal fluid flowing from the cisterna magna to the cisterna pontis and cisterna medullae oblongatae to the cisterna interpeduncularis and basalis and from there to the cisterna of the sulcus Sylvii and on to the cerebral protuberance (Kautzky & Zölch, 1953) prevents the rapid permeation of the isotope from the cisterns of the base of the cranium to the cisterna magna.

It reaches the cisterna magna, however, when, after death, the walls of the cisterns collapse and the flow of the cerebrospinal fluid is arrested or when greater pressure is employed when administering the isotope in living animals (animals Nos. 5 and 6).

The low activity of the serum is connected with the small surface of exchange.

ZUSAMMENFASSUNG

Die Verfasser bestätigten Schreiners Beobachtung, dass das in die Perilymphe des einen Ohres applizierte Isotop in die Perilymphe des anderen Ohres durchdringt und die Aktivität der Perilymphe des zweiten Ohres die Aktivität des Liquors aus der Cisterna cerebellomedullaris und des Serums übersteigt. Gleichzeitig verfolgten die Verfasser den Weg des Isotops, den die subarachnoidalen Räume und Zisternen der Schädelgrube bilden. Die niedrige Aktivität des Liquors der Cisternae cerebellomedullaris scheint durch die Tatsache erklärt zu sein, dass der Liquor in diesem Raum vor allem aus dem 4. Ventrikel stammt, den das Isotop auf seinem Weg entlang den Räumen der Schädelgrube umgeht. Die Liquorstrombahn lässt das schnelle Durchdringen des Isotops in die Cisternae cerebellomedullaris nicht zu. Die niedrige Serumaktivität kann mit der kleinen Fläche des Austausches verbunden sein.



FIG. 1 Photograph of the posterior and medial cr nial fossa of cat.

TABLE 1

Animal no.	Activity no. of impulses (10 mg/1 min)				
	C.S F from the region of cochlear aqueduct opening—first ear	C.S F from c ponto-cerebellaris—first ear	C.S.F from c. ponto-medullaris	C.S F from c interpeduncularis	C.S F from c. basilis near n.V
I	—	4850	112	—	—
II	315	400	490	—	—
III	810	700	477	192	122
IV	—	1440	2620	184	290
V (dead)	750	3150	11?0	—	480
VI (dead)	—	4?00	3700	2870	164

	C.S F from c po to-cerebellaris—opposite ear	C.S F from the region f cochlear aqued ct opening—opposite ear	Perilymph from pposite ear	C.S F from cerebello-med llaris	Serum
I	20	—	0	1	25
II	80	5	28	1	26
III	112	183	20	12	18
IV	2100	—	56	6	40
V (dead)	1070	580	240	40	—
VI (dead)	1180	550	140	0 0*	—

The isotope was administ red twice nder greater pressure thus causing the isotope to enter c cerebello-medullaris and c. interpeduncularis.

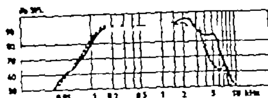


Fig. 1 —— frequency response curves for insert-earphone and – – – – external earphone measured on 2 cc coupler (IEC ref.)

METHODS

The material comprises 13 ears involving 8 persons between the ages of 23–43 years. It proved extremely difficult to obtain even this small minority for the simple reason that an acrylic earmould with builtin receiver of the type available necessitates an auditory meatus of very large dimensions. Apart from this the meatus and eardrum were normal. Persons with conductive deafness were eliminated. There were however a few cases showing a slight dip at 4000 Hz as a result of noisetrauma.

Sound Pressure Measurements

Apparatu and procedure

Individually-cast earmoulds of hard material (acrylic resin) were used for both earphone systems concerned. In the one system a wide-range type external earphone was fitted onto the ordinary earmould, which system is hereafter called external earphone. In the other system (hereafter called "insert-earphone") a special "Knowles"-type earphone was incorporated into the very tip of the earmould. Fig. 1 shows the frequency responses of the two earphones systems measured on a hard 2 cc coupler (ref. IEC).

During the sound pressure measurements in the auditory canal, the earphone was driven by a constant output from the frequency oscillator ... continuou ly ran through the required frequency range from 100 Hz ... 10 000 Hz. The sound pres ure thus produced in the meatus was measured by mean of ... probe tube aggregate (Fig. 2). The probe tube, which connects the mid-section of the auditory canal with the sound registering microphone consists of a polyethylen tube with an inner diameter of 0... mm and a length of 50 mm. The probe tube is connected to the microphone of the measuring instrument as shown in the figure. The probe tube outer end leads to a central channel into a conical top mounted on the registering condenser microphone mounted onto a long and flexible goose-neck whereby it can easily be connected to the ear. This microphone is connected through an amplifier to a recorder and from the curves thus obtained it is possible to calculate the exact sound pres ure at the different

long and narrow the acoustic impedance will be especially manifest at high frequencies the so-called "low pass filter action The volume of the chamber is of importance in that a reduced volume will increase the sound pressure effect caused by the earphone on the eardrum

Reports on the significance of the earmould have been made by a number of authors Güttner & Starke (1954) and Dalsgaard Johansen & Chisnall (1966) studied the problem by measuring the frequency response on a hard coupler Schmitt & Zehm (1966) on a hard model with dimensions similar to those of the human ear Ewertsen Ipsen & Scott Nielsen (1957) used both hard coupler and the human auditory meatus. They all found that the acoustic characteristics of the earmould are influenced in an important way by the sound channel itself which should be as short and wide as possible As pointed out by Dalsgaard Angaard Johansen & Chisnall (1966) a radical amelioration of this factor is difficult with the materials currently used without endangering the stability of the earmould and the perfect fitting in the auditory canal A reduction of the total volume with a view to increasing the sound pressure level cannot be done for practical reasons, because the effect is counteracted by the inevitable lengthening of the earmould and sound channel with the consequently weaker high pitch response

Through sound pressure measurements in the human auditory meatus Krarup & Scott Nielsen (1965) have compared the influence of the three most commonly used types of earmould on a hearing aid s frequency response The types concerned were (1) the prefabricated hard earmoulds, (2) the prefabricated soft earmoulds ("nipple type) available in different sizes and (3) individually-cast earmoulds of hard material They found that the individually-cast earmould was the best The hard prefabricated earmoulds mainly caused a loss in the lower frequencies because of the bad fit in the auditory canal The soft nipple" type caused a loss in the high frequencies, because the plastic tubing attached to the nipple acted as a low pass filter This tube showed a length of 50 mm, which is equal to the tube length of the concealed earmould connected to a "behind the ear aid If this system was used for body aids, the much longer tubing involved could cause an even less high pitch response.

The aim of this investigation was to compare two different systems of earphones First a comparison was made between the sound pressure curves which the two earphone systems produce in the human meatus, second the threshold levels of the two earphone systems were compared through Békésy audiometry

Based on the results of the above mentioned authors, an insert-earphone is supposed to extend the frequency range of a hearing aid especially in the high frequencies since the "low pass filter action in the ordinary earmould s sound channel is avoided A reduction of the total volume between earphone and eardrum should bring about a higher sound pressure level with the same earphone output



Fig. 1 —— frequency response curves for i sert-earphone, nd - - - - external earphone measured on 2 cc coupler (IEC ref.)

METHODS

The material comprises 13 ears involving 8 persons between the ages of 25–4 years. It proved extremely difficult to obtain even this small minority for the simple reason that an acrylic earmould with builtin receiver of the type available necessitates an auditory meatus of very large dimensions. Apart from this the meatus and eardrum were normal. Persons with conductive deafness were eliminated. There were however a few cases showing a slight dip at 4000 Hz as a result of noisetrauma.

Sound Pressure Measurements

Apparatus and procedure

Individually-ca t earmoulds of hard material (acrylic resin) were used for both earphone systems concerned. In the one system a wide range type external earphone was fitted onto the ordinary earmould which system i hereafter called external earphone. In the other system (hereafter called "insert-earphone) a special Knowles"-type earphone was in corporated into the very tip of the earmould. Fig 1 shows the frequency responses of the two earphones systems measured on a hard 2 cc coupler (ref IEC)

D ring the sound pressure mea ur ments in the auditory canal, the earphone was driven by con tant output from the frequency oscillator a it continu usly r n through the required frequency range from 100 Hz 1 10 000 Hz. Th sound pres ure thu produced in the meatu was measured by mean f a probe tube aggregate (Fig. 2) The probe tube which connect the mid-section of the auditory canal with the sound registering micr phone con i t of a polyethylene tube with an inner diameter of 0 mm nd a length of 50 mm The pr be tube is connected to the microphon of the measuring in truments as sh wn in the figure The probe tube outer end lead to a central channel into a conical top mounted on the regist ring condenser microphone mounted onto a long and flexible goose-neck wher by it can easily be connected to the ear This microphone i connected through an amplifier to a recorder nd from the curves thus bt ined it is possible to calculate the exa t sound pressure at the different

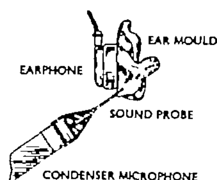


Fig. 2

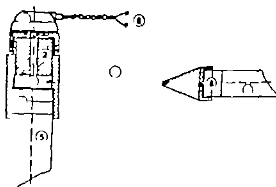


Fig. 3

FIG. 2. Probe tube aggregate for t sting sound pressure in auditory meatus, see text.

FIG 3 Set up for measuring attenuation of probe tube 1 earphon 2 earmould sub titute 3 2 cc cavity 4 condenser microphone cartridge I 5 cathod f llower 6 connecti n to beat frequ cy oscillator 7 sound probe 8 condenser microphone cartridge II 9 cathode follower

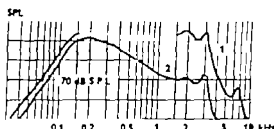


FIG. 4 Correctio curves fo atte nation in actual probe tube Curv 1 frequency response curve m asured directly n 2 cc coupler with ut earmould, cond nser microphone I Curv 2, measured on 2 cc coupler through ctual probe tube condenser microph n II

frequencies, as one corrects the loss of acoustic energy caused by the passage of the sound through the probe tube The correction values are found by recording the response curves of the earphone used both through a 2 cc coupler to condenser microphone I and via the probe tube to con denser microphone II (Figs. 3, 4) At frequencies above 4000 Hz, however these curves could not be used and for frequencies of 4000 5000 and 6000 Hz, correction was made through selective measurements by a Radiometer Wave Analyser type FRA 2 It was not possible to register the sound pressure in the auditory canal for frequencies over 6000 Hz.

Results of sound pressure measurements

An average curve is worked out for both earphone systems (Fig 5) Sound pressure is given in absolute SPL (2×10⁻⁴N/m) A direct compa rison of these curves with those measured on the hard coupler in Fig 1

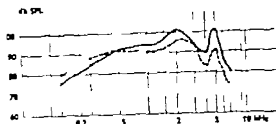


Fig. . Sound pressure lev l mea ured in uditory canal ——— average alues for i sert-earphone nd — — — for external earphon

i only of relative value on account of the differing impedance conditions. The sound pressure curves for both earphone systems measured in the au lit ry meatus can however be compared. Above 3000 Hz the curve for the insert-earphone is the highest, so that at 4000 5000 and 6000 Hz the differences are 7 10 and 10 dB respectively This is, among other factors, related to the "low-pass" filter action of the ordinary earmould's channel.

Threshold Measurements Through Békésy Audiometry

It might be possible that the relationship between the sound pressure curves could be reproduced in a pure tone audiogram through determina tion of the threshold levels. To investigate this matter studies were under taken with Békésy audiometry using the same subjects and earphone systems in direct continuation with the sound probe measurements

Apparatus and procedure

Békésy audiometer Grason Stadler Type E 800-4 was used, but its TDH 39 earphones were replaced b y the two earphone systems previously men tioned. The input power to the earphones was adju ted to ensure that both gave the same sound pressure at 1000 Hz. The pure tone signal was interrupted at the rate of 2.5 Hz and attenuated at the rate of 2.5 dB/sec using fixed frequency tracing at 125 250 Hz up to 8000 Hz as given in Fig 6

Th threshold level per frequency for the individual ear was worked out as n average of about 10 full pikes in the tracing. We have chosen to use th middle v lue f the amplitude By means of a simple correction for the earphones used the registered curves can be worked out to a precise SPL valu after comparative measurement (Fig 6) It should be pointed out th t w have been working with the threshold level during these in estigations, nd therefore with quite different sound pressure level th n those used during the probe in estigations of sound pressure in the uditory meatus.

F r both the sound pres ure mea urement and the Békésy tracing all

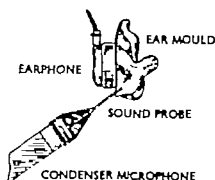


Fig 2

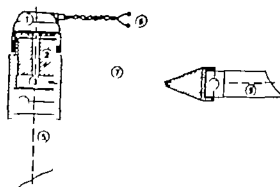


Fig. 3

FIG. 2 Probe tube aggregate for testing sound pressure in audit ry meatus, see text.

FIG. 3. Set up for m asuring att nuati n f probe tube 1 rphon 2 earmould substitute 3 2 cc cavity; 4 condenser microphone cartridge I 5 cathod follower 6 connection to beat frequency oscillator 7 s und probe 8 condenser microphone cartridg II 9 cathod f llower

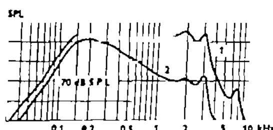


FIG. 4 Correction curv s for tte nati n in actual probe tube Curve 1 frequency response curv me sured directly on 2 cc coupler with ut earmould, conde ser microphone I Curve 2, measured 2 cc coupler through ctual probe t be condenser microph e II

frequencies, as one corrects the loss of acoustic energy caused by the passage of the sound through the probe tube The correction values are found by recording the response curves of the earphone used, both through a 2 cc coupler to condenser microphone I and via the probe tube to condenser microphone II (Figs 3 4) At frequencies above 4000 Hz, however these curves could not be used and for frequencies of 4000 5000 and 6000 Hz, correction was made through selective measurements by a Radiometer Wave Analyser type FRA 2 It was not possible to register the sound pressure in the auditory canal for frequencies over 6000 Hz.

Results of sound pressure measurements

An average curve is worked out for both earphone systems (Fig 5) Sound pressure is given in absolute SPL (2×10^{-4} N/m²) A direct comparison of these curves with those measured on the hard coupler in Fig 1

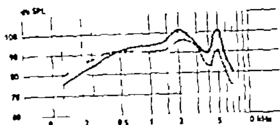


Fig. 5. Sound pressure levels measured i auditory ca al. —— verag lues for seri-earphone nd — — — for external earphone.

is only of relative value on account of the differing impedance conditions. The sound pressure curves for both earphone systems measured in the auditory meatus can however be compared. Above 3000 Hz the curve for the insert-earphone is the highest so that at 4000 5000 and 6000 Hz the differences are 7 10 and 10 dB respectively This is, among other factors, related to the low-pass" filter action of the ordinary earmould s channel

Threshold Measurements Through Békésy Audiometry

It might be pos ible that the relationship between the sound pressure curves could be reproduced in a pure tone audiogram through determination of the threshold levels. To investigate this matter studies were under t ken with Békésy audiometry using the same subjects and earphone ystems in direct continuation with the sound probe measurements.

Apparatu and procedure

Békésy audiometer Grason Stadler Type E 800-4 was used, but its TDH 39 earphones were replaced by the two earphone systems previously men tioned The input power t the earphones was adjusted to ensure that both gave the same sound pressure at 1000 Hz. The pure tone signal was interrupted at the rat of 2.5 Hz and attenuated at the rate of 2.5 dB/sec using fixed frequency tracing at 12.. 250 Hz up to 8000 Hz as given in Fig 6.

The thresh ld level per frequency for the individual ear was worked out a an verage f about 10 full spikes in the tracing. We have chosen to use the middl value of the amplitude By means of a simple correction for the earphones used, the registered curves can be worked out to a precise SPL alue after comparative measurements (Fig 6) It should be pointed out that we have been working with the threshold level during these in estigations, and therefore with quite different sound pressure level th n those used during the probe investigations of sound pressure in the udit ry meatus.

For both the sound pressure measurement and the Békésy tracing all

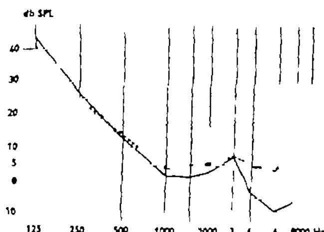


FIG 6 Threshold levels measured through Békésy audiometry ——— average values for insert-earphones, and — — — for external earphone

tests were carried out twice at intervals varying from a few days up to several weeks. Sufficient agreements were found between the tests, both for the individual ear and for the average curves

Results of threshold level tests

The curves in Fig 6 reflect those for the sound probe measurements in Fig 5 especially that the effect of the insert-earphone increases in comparison with that of the external earphone for the higher frequencies, and at 6000 and 8000 Hz is improved by approximately 13 dB

DISCUSSION

A comparison between the frequency response of an earphone as measured on a hard coupler and in the auditory canal with the actual impedance conditions of the human ear has only limited value The sound pressure curves in the auditory canal for the two earphone systems can be compared since both earphones are submitted to the same constant supply and both curves are taken with the human ear as the acoustic load. Average curves for threshold levels in Békésy audiometry may be mutually compared for both earphone systems and may also be compared with the probe tube measurements It might have been desirable to use the same levels for probe tube investigations as for Békésy audiometry but this has not been practicable, because the threshold levels are not high enough for the technique applied at the sound probe measurements

In the sound pressure chambers considered it might be possible to obtain by means of an insert-earphone (1) A reduction of the remaining volume between the tip of the earmould and the eardrum with a consequently increased sound pressure level for the same output from the

earphone (2) the avoidance of the ordinary earmould's sound channel and its "low pass" filter action. As concerning the volume, the conclusion was soon reached that it would be difficult to obtain a radical reduction of volume. First of all, it is difficult, with the accessible insert receiver and earmould material to make it small enough to be placed even in a broad auditory canal. Moreover a clinical application had to be considered, and the demand respected that an earmould must not penetrate deeper into the meatus than the cartilaginous section. The result of the investigation shows also that by use of this insert-earphone no essential increase of the sound pressure level has been obtained which might be ascribed to a reduction in volume. On the other hand, the results show a change in the form of the curve with an increase of amplification in the frequency range above 3000 Hz, proof among other things of the elimination of the "low pass" filter action, which can be imputed to the sound channel in the ordinary earmould.

CONCLUSION

Both with the sound pressure measurements in the auditory meatus and with the threshold findings of the Békésy audiogram the insert earphone showed insignificant loss of a few dB at frequencies below 500 Hz in comparison with the external earphone. On the other hand, the insert earphone produced greater sound pressure about 10–13 dB, and thus greater effectivity at frequencies above 3000 Hz.

Generally it is considered an advantage to get more amplification at the high frequencies, and insert-earphones should presumably give better results than the ordinary type of earphones. No tests are however available to show whether this condition will cause increased speech discrimination when using a hearing aid.

With the insert-earphones available at present, however this fact will hardly be of much significance for the time being. Only a small number of hard-of hearing persons will be able to use insert earphones, as these require very wide auditory canal for insertion. They require painstaking hygienic care of the auditory meatus and cannot be used by persons with secretion in the meatus. The slightest impurity on the insert earphone will have an immediate effect on its function. Contrary to external earphones, the insert-earphones cannot be separated and effectively cleaned. To cover the face of the insert-earphone with rubber coating involves practical difficulties. Furthermore the manufacturing of such a rubber coating is extremely difficult without affecting the sound pressure.

ZUSAMMENFASSUNG

Der Frequenzgang eines gewöhnlichen externen Hörers, der an ein Ohrpassstück gekoppelt ist, wurde mit einem speziellen „Inserthörer" verglichen teils durch Sondenmessungen des Schalldruck im Gehörgang und teils durch Mes-

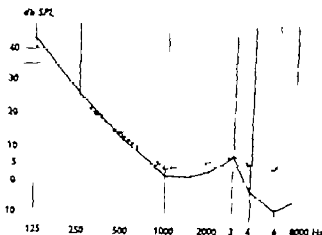


FIG. 6 Threshold levels measured through Békésy audiometry ——— average values for *insert-earphones, and* – – – *for external earphone*

tests were carried out twice at intervals varying from a few days up to several weeks Sufficient agreements were found between the tests, both for the individual ear and for the average curves

Results of threshold level tests

The curves in Fig 6 reflect those for the sound probe measurements in Fig 5 especially that the effect of the insert-earphone increases in comparison with that of the external earphone for the higher frequencies, and at 6000 and 8000 Hz is improved by approximately 13 dB

DISCUSSION

A comparison between the frequency response of an earphone as measured on a hard coupler and in the auditory canal with the actual impedance conditions of the human ear has only limited value The sound pressure curves in the auditory canal for the two earphone systems can be compared, since both earphones are submitted to the same constant supply and both curves are taken with the human ear as the acoustic load Average curves for threshold levels in Békésy audiometry may be mutually compared for both earphone systems and may also be compared with the probe tube measurements It might have been desirable to use the same levels for probe tube investigations as for Békésy audiometry but this has not been practicable, because the threshold levels are not high enough for the technique applied at the sound probe measurements.

In the sound pressure chambers considered it might be possible to obtain by means of an insert-earphone (1) A reduction of the remaining volume between the tip of the earmould and the eardrum with a consequently increased sound pressure level for the same output from the

Ac ola-laryngologica 64 237 291 (1967)

THE ULTRASTRUCTURE OF THE OLFACTORY EPITHELIUM OF THE GUINEA PIG

A AASTILA and J WERSÄLL
Turku Finland and Stockholm Sweden

From th Department f Anatomy University f Turku Finland and the Departm nt f Otolaryngology Kar li ka Sjukhuset and King Gustaf V Research Institute Stockholm

The lfactory mucosa f the guinea pig wa studied by electron microscop Three cell types, namely upporting cells, basal cells, nd recept r cell were bserved Th characteristic feat re of the supporting ll was the abundance of various single-membrane limited in lusion bod es. Centri les were observed in the supporti g c lls, but n cilia These cells did not tot lly surrou d the recept r dendrites, nd up to four dendrites could be seen l ing ide by ide Tight junction were noted between these adjacent dendrites. Tw mai types of receptor cell were l tingu shed according t the length f the de drite In th sh r test, flask l k receptor cells th dendrit w s entirely lacking. In the termi l swell ngs f the lend ites ki ocilia were observed which had t pic l basal bod thicker pr ximal shaft and a thinne distal shaft l the t ps of th ilia and in the lateral projection numerous small esicles were found Shorter ila without the distal shaft were also so ll seen Th presence of these short ila s taken s indicati n f the co nt nuou f rm tio f th cilia. The tight juncti n are con idered t be the sites f the i termittent n hronous ell lls in the *groups* of recept rs, where the l rs esicles re regarded as toring the subst nces ecessary for the depolarization f the plasma membran

INTRODUCTION

E xten i studies ha e been mad of the light microsc pic structur f the ol t ry p it elium f rodent (cf K lmer 1927 Le Gros Clark, 1956 The ecurren f three diff rent types f cell in the epithelium has been confirmed with lectr n microscopic studies and a mor detailed analysi f th ultra tructure of some nimal ha been p rf rmed (Bl m, 1954 D l r ne 19 7 1963 Reese 196 Graziadei 1965 Andres 196 1966) l these st dies great s ilaritie n tr ucture h wever have been described between d ffe rent pecies. Guin a pigs h been wid ly used a xperi n nt l animal f r ph s iological and m rpho l gical investig ti n n sense n S f r n electron microscopic tuds ha bee publ hed on th fine st ucture of the lfact ry muc sa of th s nim ls. The purpose f thi t ly i t provide morph l ogical backgr und f r f ther experiment l tig tion int the olfact ry p it li m

sungen des Schwellenniveaus bei der Békésy Audiometrie. Der Insert-Hörer gab einen grösseren Wirkungsgrad 10–13 dB im Frequenzbereich über 3000 Hz. Dies ist u. a der Aufhebung der Tiefpass" Wirkung in dem Schallkanal des gewöhnlichen Ohrpassstücks zu verdanken Eine Vergrösserung des Schalldruckniveaus, der eine Reduktion des Restvolumens im Gehörgang zugeschrieben werden kann wurde nicht gefunden Aus praktischen Gründen wird es auch schwierig sein, eine radikale Reduktion dieses Volumens mit den zugänglichen Materialien zu erreichen Die Bedeutung der besseren Diskant Wiedergabe des Insert Hörers wird diskutiert und die praktischen Schwierigkeiten der Verwendung werden erwähnt

REFERENCES

Dalsgaard, S C., Angaard Jorg sen, P and Chisnall, L. G 1966: Über den Frequenzgang v n Ohrpassstück n *Z Hörg räte Akustik* 5, 126

Ewertsen H W Ipsen, I B., and Scott Nielsen S., 1957: On acoustical characteristics f the earmould *Acta Otolaryng* (Stockh.) 47 312.

Güttner, W and St rke, C., 1954: Über die ober Grenzfrequenz von Hörgeräten. *Acusti a* 4 155

Krarup B., and Scott Nielsen S 1965 S und pressure measurements in the auditory canal (In Danish) *Nord Audiologi* 4 90

Schmitt H G and Zenn S 1966: Über den Einflu s des Ohrpassstückes uf den Frequ nzgang der Schallübertragung vom Hörer zum Trommelfell (Modelversuche an kunstlichen Oh) *A ta Otolaryng* (Stockh.) 61 121

Stat Hearing R habilitati n Centre
C penhagen, Denmark

Received January 11 1967

Acta oto-laryngologica 64: 187-194 (1967)

THE ULTRASTRUCTURE OF THE OLFACTORY EPITHELIUM OF THE GUINEA PIG

A. ANTTILA and J. WERSÄLL
Turku, Finland and Stockholm, Sweden

From the Department of Anatomy, University of Turku, Finland, and the Department of Otolaryngology, Karolinska Sjukhuset and King Gustaf V Research Institute, Stockholm

Th olfactory mucosa f th guinea pig was studied by electron microscopy. Three ell types, namely supporting cells basal cells, and re ceptor cell were bserved. The characteri ti feat re f the supporting cell wa th bundance f various ingle-m mbra e-limited inclusion bodies. Cent l les were bserved in the supporting cells, but n illa. These cells did not t t lly surround the receptor dendrites, and up t four dendrites could be seen lying sid b sid. Tight junctions were noted between these djacent dendrites. Two main types of recept r cells were distinguished according t th length of the dendrite. In the shor test, flask-lik recept r cells the dendrit wa entirely lacki g. In the term nal swellings f th dendr tes kinocilia were observed which had typical basal body thicker pro imal shaft a d a thinner distal shaft. I the t ps f th ilia and n th lateral projection numerous small es les were found. Shorter ilia without th distal haft were also usuall seen. Th presence of these sh rt cilia is t ken s indic ti n of th continuo formation of the ila. The tight j nctions are considered t be the sites f th intermittent ynchronou acti ity in the groups of receptors, whereas the ciliary esi les are regarded s stori g the substances ecessary for the depolarization f th plasma membra

INTRODUCTION

E ten iv studies hav been made of the light microscopic tructure of th olf t ry epithelium of rodents (cf. holmer 1927, Le Gro Clark, 1956). The occurrence f three differ nt types of cells in the epithelium has been confirm d with electron mi roscopic studies and m re detailed analysis f the ultra tructure of some nimal has been performed (Bloom 1954, De Lore zo, 19 7, 1963, Reese 1965, Graziadei 1965, Andres, 1965, 1966). I th se studies great variations in structure howev have been described between different species. Guinea pigs have been wid ly used a experi m ntal nimals for physiological and morph l gical investigation on sense rga So fa n lectron mi roscopi tudy ha been pub li hed on th fine tructure of the lfact ry mucosa f these animals. The purpose of this study i to pro ide a morphological background f further xperimental n estigation into the olfact ry epithelium

sungen des Schwellenniveaus bei der Békésy Audiometrie Der Insert-Hörer gab einen grösseren Wirkungsgrad 10–13 dB, im Frequenzbereich über 3000 Hz. Dies ist u a der Aufhebung der „Tiefpass" Wirkung in dem Schallkanal des gewöhnlichen Ohrpassstücks zu verdanken Eine Vergrösserung des Schalldruckniveaus, der eine Reduktion des Restvolumens im Gehörgang zugeschrieben werden kann, wurde nicht gefunden Aus praktischen Gründen wird es auch schwierig sein, eine radikale Reduktion dieses Volumens mit den zugänglichen Materialien zu erreichen Die Bedeutung der besseren Diskant Wiedergabe des Insert-Hörers wird diskutiert und die praktischen Schwierigkeiten der Verwendung werden erwähnt.

REFERENCES

Dalsgaard, S. C., Angaard Johansen, P., and Chisnall, L. G., 1966: Über den Frequenzgang von Ohrpass tücken *Z Hörger (Akustik 5 126*

Ewertsen H W Ipsen, I B., and Scott Nielsen S 1957: On acoustical characteristics f the armould *Acta Otolaryng* (Stockh) 47 312

Güttner, W and Stahre, C., 1954: Über die obere Grenzfrequenz von Hörgeräten. *Acustica* 4 155

Kraul B., and Scott Nielsen S 1963 Sound pressure measurements in th auditory canal (In Danish.) *N rd Audiologi* 4 90

Schmitt H. G and Zehm S 1966: Über den Einflu s des Ohrpassstückes auf den Frequenzga g der Schallübertragung vom Hörer zum Tromm lfell (Modelversuche am künstlichen Ohr) *Acta Otolaryng* (Stockh.) 61 121

State Hearing Rehabilitation Centre
C penhagen D nmark

Received January 11 1967



F 2 A survey picture of the olfactory epithelium. The receptor cell (RC re distinguished by their round nuclei and conspicuously large nucleoli. Most of the nuclei of the re ptor cell re situated more basally than those of the support g cells, xcept one lort cell ith the nucleus nea the free border f the epithel m. 3000)

yl ndrical supporting cells are most numerous and their oval nuclei are usually ituated near the free surf ce of the epithelium. Th receptor cells re it ted between the supporti g cells, and their nuclei are usually loc ted m re basally than the upporting cells. However there i lso a fl k l ke type f the recept r cells, the nuclei of which are situated more pie lly than the nuclei f the supporting cells. In both the light and the lectron microscopic section these cell re easily distinguished from the pporting cell by th ir round nuclei which are provided with a large, round ncleolus in the center

The basal cell are seen near the basement membran as small, round r polygonal cells, which have little cyt pla m around the nucleus.

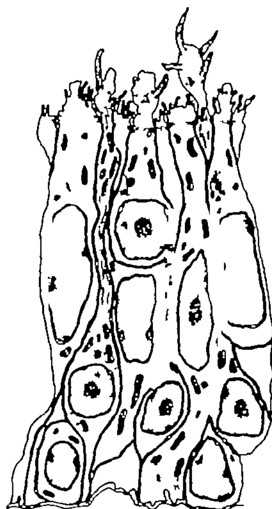


Fig 1 A schematic drawing of the olfactory epithelium

MATERIAL AND METHODS

Ten guinea pigs, weighing from 400–600 g, were killed by rapid decapitation The olfactory mucosa was first fixed *in situ* with cold 1% osmium tetroxide buffered with phosphate buffer to pH 7 2 7 4 (Millonig 1961) for 10 minutes, and subsequently removed and fixed in a refrigerator for three hours. The specimens were washed and dehydrated in the usual manner and embedded in Epon 812 (Luft 1961) The sections were cut *with an LKB Ultrotome and stained with uranylacetate (Watson 1958)* and lead citrate (Reynolds, 1963) was employed Photographing was done with Siemens Elmiskop I electron microscope using the acceleration voltage of 60 KV

The General Architecture

The pseudostratified olfactory mucosa is composed of three different cell types, namely supporting cells, olfactory receptor cells, and basal cells (Figs 1 and 2) All these cells rest on a thin basal membrane The long,



Fig. 2 A survey picture of the olfactory epitheli m The receptor cell (RC) are distinguished by thei round uclei and con picuously large ucleoli. Most of the uclei f the receptor cell re situated more basally tha those f the supporti g cells, except on l ort cell ith the nucle nea the free border f the epithel m. 3000

cylindrical upporting cells are most numerou and their oval nuclei are u ually situated near the f ee surface f the epithelium The receptor cells are ituated between the upporting cells, and their nuclei are usually located more basally than the supporting cells. However there is also a fl k-like type of the recept r cells, the nuclei of which re situated more pically than th nuclei of the supporting cells. In both the light and the electron microscopic section these cell re easily disti guished from the pporting cells by their round nuclei which are provided with a large rou d nucleolus in the center

The basal cells re seen near the basement membrane small round or polygonal cells, which have little cytoplasm round the nucleus.

The supporting cell

This cell extends from the basement membrane up to the free surface of the mucosa. For presentation this cell can be divided into the foot-like process, which extends from the basement membrane to the nucleus, the cell body around the nucleus, and the apical portion of the cell which reaches the free surface of the mucosa and extends outside the surface forming club-shaped extensions (Fig. 3).

The cytoplasm of the basal process is composed of long mitochondria, free ribosomes, and granular endoplasmic reticulum. A few single-membrane limited inclusions (SMLIB) resembling lysosomes can also be seen in this part of the cytoplasm. As a whole, this part of the cytoplasm has fewer organelles than the apical and middle parts. The nucleus of this cell type is large and oval and its distance from the surface of the epithelium is shorter than its distance from the basement membrane. The nucleus is rich in chromatin material, which is arranged in big clumps near the nuclear envelope. The nucleolus, however, is quite small compared with that of the sensory cell. The cell body is composed almost entirely of the nucleus, and on its side there is only a little cytoplasm. The apical cytoplasm has abundantly organelles. The Golgi complex is usually large and may extend near to the surface of the cell. There are many elongated mitochondria which are usually arranged parallely to the long axis of the cell. Both granular and agranular endoplasmic reticulum and free ribosomes can be seen. In addition there are two types of single-membrane-limited inclusion bodies, one resembling cytosomes and the other multivesicular bodies.

The plasma membrane of the surface forms numerous microvilli and usually a larger extension which is situated in the middle of the microvilli and continues beyond the surface of the mucosa. The size and shape of the microvilli vary very widely. Most of them are long and thin whereas others may be thicker and divide into secondary branches. In material fixed with osmium tetroxide the whole microvilli can be seen throughout as thin filaments. In the thicker microvilli free ribosomes are observed and sometimes there are small vesicles in the proximal parts of the microvilli. The same kind of coated and noncoated vesicles are seen abundantly between the microvilli just under the plasma membrane (resembling micropinocytotic vesicles). The cytoplasm inside the club-shaped extension is usually devoid of organelles, except ribosomes. Sometimes, however, a few vesicles can also be seen in the extensions. No cilia originating from this cell can be seen, but one or two centrioli are frequently observed in the apical cytoplasm near the surface plasma membrane. In the plasma membrane joining one supporting cell to another or to the dendrite of the sensory cell three types of specialized membranes can be observed. Nearest to the free surface is the tight junction or zonulae occludentes. Proximal to tight junctions there are intermediate junctions or zonulae adhaerentes. Below this area desmosomes can be seen with dense fibers crossing the whole neck



Fig. 3. A pporting cell with ov l nucleus, with bo dant chromatin m terial dja- cent t the lear em lope I the ytopla m merous agranula esicles. I the free border of the cell microvilli and larger extension of the cytoplasm. 12,000

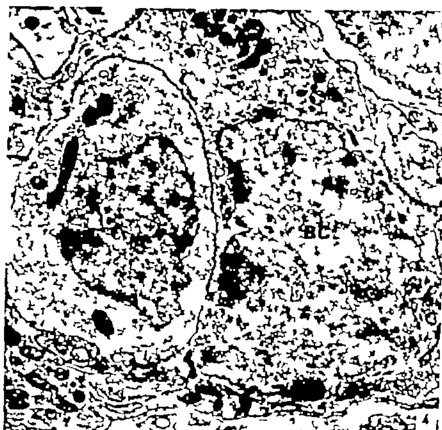


Fig. 4. Two basal cells (*BC*) lying on the basement membranes. In the nuclei abundantly l m tin d interchromatin granules are visible. In the cytoplasm there are numerous free ribosomes. × 12,000

of the supporting cell. Interdigitations are also frequently seen between the supporting cells and the receptor cells.

The basal cell

These cells are small polygonal cells which lie on the basement membrane. The cytoplasm and nucleus of these cells are rather dark and can be easily distinguished from the pale processes of the supporting cells.

The ovoid nucleus is situated in the center of the cytoplasm. It has fairly small nucleoli and large clumps of chromatin material supported by the nuclear envelope in the same way as in the nucleus of the supporting cells (Fig. 4). This interchromatin area is, however, rich in particles which gives the nucleus its dark appearance.

The cytoplasm is composed of small mitochondria, Golgi complex, and granular endoplasmic reticulum. The dark appearance of the cytoplasm is mainly due to free ribosomes, which are abundant and form small rosettes. These cells have also thin processes in which mitochondria, granular endoplasmic reticulum, and free ribosomes can be seen.

The olfactory receptor cell

In order to facilitate description this cell will be divided into the following parts the *olfactory axon* which extends from the cell body to the lfactory bulb but can be followed in electronmicrographs down to the basement membrane the *cell body* which consists of the nucleus and the cyt plasm around it, the *dendrite* which extends from the cell body up to the surface of the mucosa and the terminal swelling which is the projection of the dendrite beyond the surface of the mucosa.

General Appearance

Most of the olfactory cells are pillar haped the nuclei of which are situated basally to the nuclei of the supporting cells (Figs. 1 and 2) A minority of the cells are short and flask-like in shape with the nuclei lying near the free surface of the epithelium, more apically than the nuclei of any other cells (Fig. 5) Th different appearance of these cells is due to the length of the dendrite portion of the cell and apparently there is a continuous series of cells from the short, flask-like cells to the long, rod like cells.

The cell body

The nucleu It is nearly round or oval has a large round nucleolus in the middle The nucleus contains very little chromatin material, which is arranged in small clumps supported by the nuclear en elope Moreover t nucleus ha rather few granules and the whole of it ha quite a pale appearance

The cytoplasm around the nucleus The Golgi complex is usually small and is situated near the envelope The cytoplasm, on the other hand, is rich in mitochondria, granular endoplasmic reticulum, and free ribosomes. There are also single-membrane-limited inclusion bodies near the nucleus wh h resemble cytosomes and multivesicular bodies. Most of the cytoplasm i it ated near the cell body where centrioles can also be seen. On the side of the nucleus there is, on the contrary only a thin layer of cytoplasm.

Th axon

This is a process from the olfact ry cell which can be followed to the basement membrane. The axon are de oid of any structure other than axon filaments nd mitochondria, which are situated indi idually among the f laments.

The dendrite

The length and structure of the dendrite vary in different cells. There are some cells in which a typical dendrite is not seen and the perikaryon extends t the terminal swelling, whereas in most receptor cells the dendrite is about three times as long s the cell body



Fı 5 A fl k lIk recept lI l kl g th d d t portl n 15 000

The long dendrite This is a narrow structure the diameter of which varies from 1 μ to 2 μ In the course of the dendrite there are occasional single-membrane-limited inclusion bodies In the thinner parts of the dendrite there are many microtubules about 150 A thick and among them are mitochondria, vesicles of agranular endoplasmic reticulum and free ribosomes In the apical part of the dendrite a cluster of mitochondria are usually seen among which the proximal centrioles are situated In many

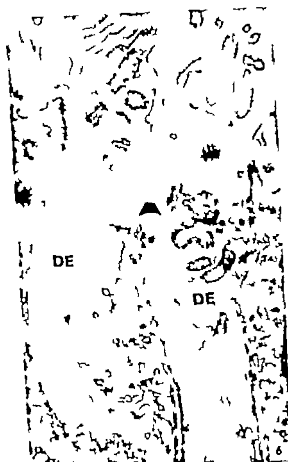


Fig. 6. A tight junction (arrow) between two adjacent dendrites (*DE*). 24,000

cases this part of the dendrite appears as a conspicuous swelling. The different dendrites are usually separated from each other by the proximal cytoplasm of the supporting cells. The apposing plasma membranes have an intracellular space about 100 Å thick, between them, except in the apical part of the dendrites. Here the contacting plasma membranes are usually separated from each other by the normal 200 Å wide, extracellular space. However there are also cases where the plasma membranes form a zonulae occludens type of junction where the extracellular space is only about 80 Å thick (Figs. 6 and 7). Usually there are only two dendrites lying side by side but in the neighbouring receptor cells up to four dendrites can be seen lying side by side.

The short dendrite. In some instances the typical dendrite portion seems to be entirely lacking and the cell body is continuous with the terminal swelling. Here no microtubules can be found between the cell body and the terminal swelling. However there are also cases where the dendrite is up

to 5 μ long but also lacks the typical pattern of microtubules and mitochondria

The terminal swelling

The structure of the terminal swelling seems to be the same in the cells of both the long and the short dendrites, except for the presence of the microtubules in the long cells. The terminal swelling is an oval structure about 2–3 μ long and 1 μ thick which extends beyond the free surface of the mucosa. Compared with the club-shaped extensions of the supporting cells the cytoplasm of the terminal swelling contains many organelles. There are mitochondria, small vesicles and granules, microtubules, ribosomes, centrioles and basal bodies, all of which form a complex but typical arrangement of the swelling, but some of them may be situated also in the apical part between the basal bodies. In the terminal swelling of the long dendrites, microtubules often continue up to the swelling, which seems to be the only difference between the terminal swellings of the long and the short dendrites.

The vesicles are of various size. Most of them are small, about 400–600 Å and are limited by a single membrane. These vesicles are seen both near the plasma membrane and around the basal bodies. In the latter case the vesicles form a ring around the basal body or there may be a circular tubule near the basal body, which is surrounded by the vesicles. In addition to small vesicles there are bigger vesicles or vacuoles which are limited by a single membrane and resemble the vacuoles of the agranular endoplasmic reticulum. There are also special types of granules with a characteristic shape. Inside their limiting membrane there is a circular dark vesicle, with a diameter of about 80 Å. The largest diameter observed in this type of granule was about 1500 Å (Fig. 7).

The plasma membranes of the terminal swelling show two kinds of special structures, the olfactory cilia and microvilli. The latter are however only occasionally seen in the sections of the swellings.

The cilia

The total length of the cilia can only be approximately estimated in electron micrographs. The length seems to vary from 2–3 μ, but there are also shorter cilia the length of which is less than 1 μ.

The cilia have two distinctive portions, the broader proximal shaft and the thinner distal shaft (Figs. 8 and 10). The diameter of the proximal shaft is about 250 mμ except on those places where there are special thickenings. The inner structure of the proximal shaft shows the characteristic pattern of nine double peripheral fibers and two central fibers. One of each of the peripheral fibers has two small arms, which are directed either towards neighbouring subfibers or towards the outer membrane (Figs. 9 and 11). A small projection can be seen in each of the central fibers. In



FIG. 7 A terminal sw lli g with tw basal bodies (*BB*) Three dense nd three light zones are distinguished i the basal foot of one them. A circula ingle-membra e-l mited structure (arrow) with circul double-membrane lamella inside The terminal swelling is connected t djacent pporting cells by tight j nction (*TJ*) Nea the sw lling oblique section of dist l shaft f the ilia (*DC*) with esicles and tw fibers. 40,000.

longitudinal sections the peripheral fibers are observed t end in the basal body forming a small exten i n, whereas the central fibers end about 300 mμ from th basal body Proximal to the endings of the central fibers there is a dense structure resembling the basal plat (Fig. 8) This plate appears t c nsist of the irregularly bent, proximal end of the central fibril and an interfibrillar dense substance At this height the diameter f the shaft narrows from 250 mμ to 210 mμ Approximately at the level of the plasma membrane, the peripheral double fibers change to triple fibers (Fig. 9) These triplet fibers are formed apparently by the addition f an e tra outer fiber t ea h peripheral double fiber Around the basal body there are one or two basal feet, which origin te from the sets of the tripl t and consist, in this case f three intermittent light and three in t rmittent dense zones (Figs. 7 and 10) Th centrioles have the same kind f triple structure a the basal body and they can be distinguished from the basal bodies only by their m e basal position in the proximal part of th terminal swelling r in the dendrite proper Al ng the proximal shaft of the cilia lateral projection are observed, in which the same kind of vesicles are situated a those f und in the terminal swellings or in the tips of the cilia.

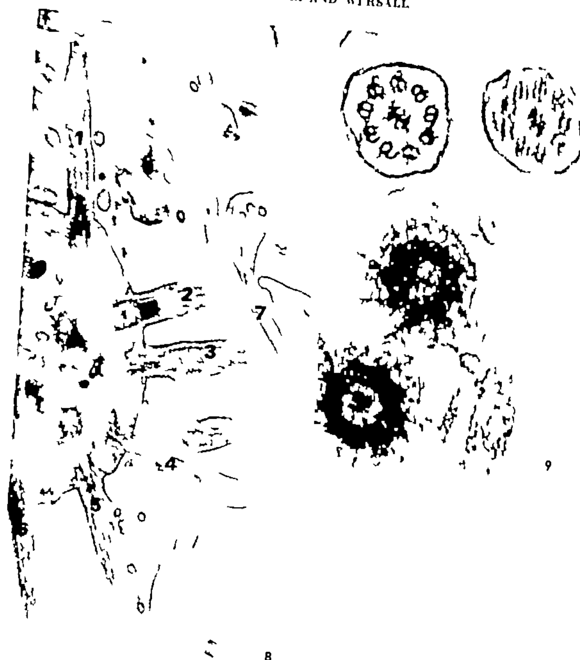


FIG 8 A t rmin l w ll l g with merou ll A l t l p jecti with esicles l ll No 5 d N 4 l git di al secti of sh rt l m with t di t l h ft A d b l pl t l seen in illum N 2 Cilium 1 h w tran iti al betwee the proxim l nd th di t l h ft N 7 l l gitudinal secti f th t p of th illum 42,000

FIG. 9 A tran rse section f pro imal ili m, howing th t pical 9+2 pattern d mall rm both in th central nd pe ipheral fibers. N t th dditi f th rd t fiber t th d bl peripheral fibers in the basal body 96,000

These lateral projections do not extend around the whole shaft of the cilia but are confined to one side of the shaft only

The vesicles of these projections are situated between the peripheral double fibers and the outer membrane of the projection



FIG. 10 A grow g cilium (GC) w th microvillus-like distal shaft Nea the lling m on tra s er sections of the distal shafts f the lia (arrows) with tw fibers the center On the left tip of cilium (TC l the t rminal sw lling merou basal bod s, one f which ha bas l foot (BF) 48,000

t di tance of 1 1.5 μ from the basal body the proximal shaft narrows t f rm 100–130 mμ thick distal haft. Howev r there are also cilia which entirely lack di tal shaft In these cases the proximal sh ft ends d re tly with r without f rming a small extension, where small vesicles seen.

The di tal hafts d not hav the typical pattern of two central fibers and ne d ubl or l gle peripheral fibers. In tead, only one or two fibers ar seen in tran erse section f the di tal sh fts. In longitudinal sections these fibers ppea t be tinuou with the central fibers f the proxim l shaft, wh rea the double peripheral fibers end at a le l with th transit ry z ne between the proximal nd dist l shaft Along the distal shaft there re the sam kind of vesi le a in th pr ximal sh ft These vesicles are

either situated between the fibers and the outer membrane or they wholly replace the fibers, so that they are the only structures in the distal shafts. The tip of the distal shaft forms an extension 300–500 mμ thick, where also vesicles are seen. Between these vesicles the two distal fibers are observed to end as thin coiling filaments.

DISCUSSION

In addition to the sensory cells two other types of cells are usually described in the olfactory mucosa: the basal cells and the supporting cells (De Lorenzo 1957, Breitschneider 1958, Graziadei, 1965, Andres, 1966). These cells, however, are distinguished from each other not only by their location in the olfactory epithelium, as has been earlier described (De Lorenzo 1957), but also by their ultrastructure indicating the different functions of these cells. Both the cytoplasm and the nucleus of the basal cells have a dense appearance depending on the relatively larger number of ribosomes and the greater amount of chromatin material which they contain in comparison with the supporting cells. The cytoplasm of the supporting cells has, on the contrary, rather a small number of ribosomes, whereas other cytoplasmic organelles, especially single-membrane-limited inclusion bodies, are a characteristic feature of these cells. The free border of the supporting cells is described as having numerous microvilli, whereas cilia have been found, electron microscopically, only in the terminal swellings of the sensory cells (Bloom 1954, De Lorenzo, 1957, Reese 1965, Andres, 1966). However, besides the microvilli, large bulb-like extensions of the cytoplasm seem to be an essential portion of the free border of the supporting cells. By light microscopy tiny flagella are described in the supporting cells as well as diplosomes (cf. Kolmer 1927). In this study no cilia or flagella originating from these cells could be found; on the other hand, one or two centrioles were seen in many supporting cells near the free border of the cell. The cytoplasm of both the sensory cells and the supporting cells contains single membrane limited inclusion bodies, but in the supporting cells they are more numerous and of various types. The lysosome-like activity of these bodies is clearly observed under experimental conditions, where they change to large bodies inside which destroyed cytoplasmic organelles can be seen, thus probably corresponding to active phagocytosis seen in these cells by light microscopy (Le Gros Clark, 1956).

In light and electron microscopic reports the olfactory receptor cells have been described as completely surrounded by the supporting cells, except for the terminal swellings of these cells, which lie outside the limiting margin of the epithelium (De Lorenzo, 1963, Le Gros Clark, 1956). Although this is the case with most receptor cells, a number of receptor dendrites were observed in this study to be situated side by side. Between the plasma membranes of these adjacent receptor cells there were usually intercellular clefts of 200 Å, but in a number of cases there were also tight junctions between

them Electrophysiologically intermittent synchronous activity has been described in certain groups of receptors, which is dependent on the connec tions of the olfactory bulb (Adrian, 1955 Takagi & Shibuya, 1960 1961 Ottoson, 1959) Morphologically this kind of activity has been explained s due either to the close apposition of the axons in the olfactory fascicles (De Lorenzo, 1957) or to the contact of the cilia in adjacent terminal wellings (Le Gros Clark, 1956) In this study no contacts between the cilia were found which would support the latter view On the other hand, the tight junctions between adjacent plasma membranes resemble elec tronic junctions between teleost spinal neurons (Bennet *et al* 1963) and between sensory cell and nerve chalice in the vestibular epithelium of mammals (Engström Ades & Hawkins, 1965 Wersäll & Lundquist, 1966) Because of the considerable length of the dendrites it is not possible in lectronmicrographs to determine the frequency of this kind of close con tact between the dendrites. However the possibility that the synchronous acti ity f the recept rs i partly or totally due to these contacts between the dendrites cannot be ruled out.

In earlier light-microscopic reports two or more different cell types have been described, which may be specific of different odours. Thus, Vinnikov 1956) described rod-shaped and flask shaped cells in the olfactory epithelium of man, dog, and rabbit. The receptor cells of the rabbit have also been described as arying for instance, in the length and attenuation of the rod processes s well as in the way they reacted to lesions of the olfactory bulb (Le Gros Clark, 1956) In electron microscopic reports such differences have n t been found which would justify dividing these cell into different subtypes, except for the difference in the presence or paucity of mitochondria the olf tory rod (De Lo enzo, 1957) In thi study two main types of rec pt r cells were bserved which corresponded to those described by Vin nikov (1956) although there seemed to be also int rmediate cells which uld not be assigned to either of the main types. The difference between th m seemed t depend mainly on the length and structure of the dendrite Recently similar results ha e also been btained by Andres (1966) in the olfactory dendrites of the sensory cells in the cat, rat, and dog, in which the number f microtubules seen in transection of the dendrites vary sub tantially It i now uggested that functional differences in the sensory cell d not necessarily imply morphological differences (Moulton & Tucker 1964 It seems reasonable to a sume, however that these difference in the tructure of the dendrite may affect the threshold of the depolariza t n of the recept cells, and can, theref re take part in the peripheral analyzing f the odours.

T rm nal swellings of the receptor cells have been reported by Le Gros Clark (1956 to also differ from ea h other by the number of olfactory hairs, by the size of the swelling, and by their affinity for silver stains. I this tudy the only difference between the terminal swellings of the rod-like and the fla k-like recepto cells was the lack of microtubules in

the latter which probably explains the different affinity of these swellings for silver stains.

According to De Lorenzo (1956) the cilia of the olfactory cells in the rabbit are 1–2 μ long and are structurally identical with those of the respiratory pathways. Bloom (1954) described the olfactory cilia in the frog as 6–12 μ long and with a typical fibrillar structure. The present paper demonstrates that the cilia on the olfactory sensory cells are divided into two parts, the proximal part of which has a kinocilia like appearance with fibrils grouped in the typical 9+2 arrangement and a distal shaft provided with two fibrils derived from the central fibrils of the proximal shaft. Each kinocilium is provided with a basal body with a triple-tubule arrangement and with one or two basal feet. It is interesting to note that these cilia do not form a regular pattern as in the cells of the respiratory pathways, where the basal bodies on each cell face in the same direction, but seem here to be arranged in a random pattern. Andres (1966) found two segments in the olfactory cilia of the macrostomes, but no basal bodies, although from his picture 3, it can be seen that basal bodies do exist. Reese (1965) described two parts in the olfactory cilia of the frog. The distal part of these cilia contained however only nine single peripheral fibers and two central fibers. From these studies it appears that there is a certain difference between various species in the structure of the olfactory cilia.

The fact that short cilia without a peripheral shaft as well as clusters of basal bodies without a cilium were found in some cells, indicates that the cilia of the adult animal might be formed continuously. Consequently the cilia cannot be regarded as permanent structures of the olfactory cells but as structures that can be destroyed and replaced. This seems to be of great importance for the cells if the cilia are as suggested part of the receptor structure. Located in the nose and in constant contact with mucous and bacteria destruction of the cilia seems to be unavoidable under certain circumstances, and their replacement through outgrowth from newly formed basal bodies, as indicated in the present study seems to be an essential condition for the proper function of the organ of smell. This fact does not seem to have been discussed in the previous studies. However the replacement of kinocilia in other cells has been discussed in a similar way (Roth & Shigenaka, 1964. Ewert 1965).

The most typical feature of olfactory cilia in the guinea pig is the abundancy of vesicles in the projection of the proximal shafts and in the tips of the cilia which usually form bulb-like extension. The same kind of vesicles are observed also in the cilia of the olfactory mucosa in the frog (Reese 1965) whereas in ordinary kinocilia they are either absent or their number is much smaller. Morphologically the packing of these vesicles near the plasma membrane resembles the location of the synaptic vesicles near the depolarizing plasma membrane in which case the vesicles are known to store various substances essential for the depolarization of the mem-

brane In view of this similarity in the location of small vesicles near the depolarizing membrane it can be assumed that the membrane adjacent to these vesicles functions as the primary site of olfactory sensation, and that these vesicles are an important part of the depolarizing mechanism

ZUSAMMENFASSUNG

Die Geruchsmukosa des Meerschweinchens wurd mit Hilfe eines Elektronen mikroskops studiert. Drei Zelltypen nämli h Stützzellen Basalzellen und Sinnes-zellen wurden beobachtet Die charakteristisch n Züge (Strukture) der Stütz zellen waren die grosse Anzahl von verschiedenen single-membrane-limited in clusion bodies" (SMLIB) Centri len wurden in den Stützzellen beobachtet, da gegen keine Zilien. Diese Zellen umlagerten die Sinnes-Dendriten nicht vollstän dig und man kon te bis zu vier Dendriten neben inander sehen. Man beobach-tet dicht Verbindungen zwischen diesen angrenzenden Dendriten Zwei Haupt *t pen der Sinneszellen wurden je nach der Lä ge der Dendriten unterschieden.* Die kürzesten, flaschenartigen Sin eszellen hatten k i e Dendriten, aber bildeten kugelförm ge Endschwellungen, die mit Kinozilie besetzt waren Auch die typischen Dendriten bildeten Schwellungen uf welchen Zilien beobachtet wur den Die Zilien hatten typische Grundkörper und einen dickeren proximalen und inen dünneren verlangerten Schaft In den Spitzen der Zilien und den seitlichen Verzweigungen fand ma zahlrei he klein Vesikeln Kürzere Zilien oh e distalen Schaft wurden au h bezeichnend für die unu terbrochene Formation der Zilien. Die dichten Verbindungen werden als Sitz der intermittenten, synchro isierten Tätigkeit in der Grippe der Sinneszellen gesehen während die ziliaren Vesikel \ aufspeichernd fur die zur Depolarisatio notwendigen Substanzen angesehen werden.

REFERENCES

ADRIAN, E. D 1955 Pot ntial oscillations i the olfactory organ. *J Physiol* (Lond.) *128*, 21

A wata, K. H 1965 Differenziern g nd Regeneration en Sinnesrell i der Regio olfactoria. *Naturwissenschaften 32* 600

— 1966 Der Fei ba der Regio lfactoria on Makrosmatikern. *Z. Zellf rsch 69* 146

BEN ET X \ L., ALJURE, E., NAKAJIMA, Y nd PAPP A, G D 1963 Electrotonic June tions bet een t leost spi al neurons Electrophys logy nd ltrastructure. Science *141* 262.

BLOOM, C 1954 St dies on the lfactory ep thel m of the frog d the toad with the id f light nd lectron microscopy *Z Z llforsch., 41* 89

DEMPTRACHIAN, H 1958 Elektronenmikroskopische Untersuch ngen d Nasen-schleimhaut *Anat An. 105* 104

ENGSTRÖM, H ADES, H. W and H w va, J E. 1963 The estibular sensory cell nd their innervation *Sympos II ng Biol 5*, 31

F G 1963 On the mucos flow rat the huma ose *Act Otolaryng* (Stockh.) Suppl *200* 1

G sselin, P 1963 Electron microscopic bservations the lfactory mucosa f the *at Exper! lie 21* 274

KOLMER, W 1927 Geruchsorgan. I *Handbu h d m kroak pisch Anatomie des M nschen*, 3 Teil 1 192. Berl n, Springer

Le Gros Clark, W. E., 1956 Observations on the structure and organization of ol f ctory rec ptors in the rabbit Yale J Biol Med 29 83

— 195 Inquiries into the anat mical basis of olfact ry discriminatio The F rita Lectur 116 290

Lore zo, A I de, 1957 Electron microscope observation of the olf ctory mucosa and olfactory nerv J Bi phys Biochem Cytol 3 839

— 1963 Studies on the ultrastructure and hi t physiology of membranes, erve fibers an l synaptic juncti n in chem receptors In Olfacti n and taste ed by Y Zotter man Oxf rd, London, N w York, Pa is Pergam n Press

Luft J H 1961 Impro en ent in po v re in embedding methods J Biophys. Biochem Cytol 9 409

Millonig, C 1961 Ad antages f a ph sphat buff from OsO$_4$ soluti ns in fixation. J Appl Phys l 32 16

Moulto D C and Tucker, D 1964 Electroph iology of the olfactory syst m Ann. N Y Acad S i 116 380

Ottoson, D 1959: Comparison of low potenti ls ev ked by natural stimulation Act Physl l Scand 4 149

Rhodin, T 1963 Olfactory ilia in the f og J Cell Biol 2 209

Rey olds, E. S 1963 The use f lead citrat t high pH as an electron st i in elec tron mic oscopy J Cell Bi l 1 208

Roth L. E., nd Shi gnaka, Y 1964 Th tructu e and formati n f ilia nd filament in rumen protozoa J Cell Bi l 20 249

Taka h, S F and Shibuya, T 1960 Th potenti l oscill ti ns observed in the olfactory pith lium, n rve nd bulb f th t ad nd frog Jap J Phy iol 10 499

— 1961: Studies on th pot ntial oscill tions appearing in the lfact ry pitheli m f th toad Ibid 11 23

Vin ikov Y A 1956 Structur of the rg of mell Arch Anat Histol Embriol 33, 49

W tso M L 19 8 St i ing of ti ue secti n for lectron mi roscopy with hea y metals J Bi phys Biochem Cyt l 4 475

W rsäll, J and Ft nq ist P-G Morph l gi al polarizati of th mecan receptors f the estibular nd a u t t m NASA Second S mp. n the Role of the Vestibula Org n in Space F pl rati n, p 3 NASA 1966

A Arsl la M.D Dept f Anatomy Un ver ity f Tu ku Tu ku F nland
J W rsäll M.D D pt f Ot laryngology Ka ol n ka Sjukhu t
Stockh lm 60 Swed n

R c ed January 9 196

Acta oto-laryngologica 63 204–212 (1967)

PRECISION AUDIOMETRY

M. E. BRYAN and W. TEMPEST
Salford England

From the Department of Electrical Engineering University of Salford Salford

Repeat within ear threshold variance can be considered to be made up of variables associated with the sensory system and those associated with the criterion adopted by the subject as to what is his threshold. Conventional audiometry is not able to separate out the effects of these different sources. Signal detection theory as applied to psychophysics would seem to offer a possible method of measuring the effects of the sensory and criterial variables separately. The variance in the measures of each of these terms for normal ears should be smaller than the variance of conventional threshold determinations. Hence the behaviour of normal ears can be more precisely specified and so departures from normality should be detected earlier than is at present possible. The problems which must be overcome if signal detection theory is to be successfully adapted for use in routine audiometry are discussed.

Accuracy of conventional audiometry

Table 1 shows the accuracy which can be obtained with repeat within ear MAP (minimum audible pressure) and MAF (minimum audible field) threshold estimates on clinically normal ears. The figures given are the change in threshold value in dB which must occur for the result to be significant at the 1% level of probability.

Both sets of data were obtained with intervals between tests greater than one day. A clear gain in accuracy is obtained using MAF rather than MAP procedures, particularly at 100 c/s, but both methods of determining thresholds are clearly rather imprecise.

In order that earlier diagnosis may be made of hearing disorders and of industrial noise induced hearing loss, it is clearly desirable that the accuracy with which thresholds of hearing are at present measured should be improved. To do this we must examine the factors which are responsible for the uncertainty associated with such determinations. It will then be necessary to either eliminate or control these factors.

Components of within ear variance

Hempstock, Bryan & Webster (1966) have hypothesised that within ear variance is made up of the following components

Total Variance $T^2 = \rho\sigma^2 + p^2 + \beta^2 + \alpha^2$ (1)

where

σ^2 = variance due to long term changes in acuity (for periods greater than one day)

σp^2 = variance due to uncertainty of replacement of sound field,
σj^2 = variance due to changes in subject's criterion of what is his threshold,
σt^2 = variance due to changes in apparatus and short term physiological changes

In MAP work it has been established by Robinson (1960) that the main component in repeated within ear threshold variance is σp^2 which is due to uncertainty of headphone replacement. This is confirmed by Atherley & Lord (1965 *a*) and Atherley, Lord & Walker (1965 *b*). It would seem that considerable uncertainty is inherent in this method of producing the sound field due to the variability of ear impedance and geometry. Although the latter workers suggest that the use of circumaural earphones will reduce σp^2 somewhat, it seems unlikely that a substantial improvement in accuracy can ever be achieved by such a method of applying the sound field to the ear.

Hempstock, Bryan & Webster (1966) have shown that in repeat determination of MAF the value of the replacement variance (σp^2) is not significantly different from zero. This is seen in table (2) which also shows that the terms due to changes in acuity (σa^2) and due to judgement and apparatus combined ($\sigma j^2 + \sigma t^2$) are each approximately half the value of the total within ear variance σT^2. We are thus left with components of variance which can be shown to be due to various factors. Some of these factors can be controlled and the effects of others can be eliminated.

Examination of the components of variance

The various components of within ear threshold variance would appear to arise from physical-cum physiological and psychological causes. Any examination of these components may be made from this viewpoint.

Physical-cum physiological causes

Two such causes of the variance due to long term changes in hearing acuity (σa^2) are (i) the effect of pre-test noise exposure and (ii) middle ear pressure.

It has been shown that threshold improvement of up to 10 dB can occur in normal ears after removal from low level noise (less than 70 dB) and that the amount of improvement is a function of the pre-test noise level (Bryan, Parbrook & Tempest, 1965 *a*). Improvement occurs for up to one hour and there is evidence of a possible correlation at high frequencies between the amount of threshold improvement and the changes found in long term acuity (Bryan, Parbrook & Tempest, 1965 *b*). This suggests that in order to reduce the value of (σa^2) due to previous noise exposure (which must inevitably vary from day to day) a pre-test period in a controlled noise environment is necessary. Some 10–15 minutes ought to be sufficient to produce more stable threshold values. As Bryan, Parbrook & Tempest (1965 *a*, *b*) showed thresholds improve approximately exponentially, hence

TABLE 1

Frequency (c/s)	Shift (dB) MAP (Robinson, 1960)	Shift (dB) MAP (Hempstock *et al.* 1966)
100	16	7
500	12	9
1 k	12	10
3 k	9	8
6 k	18	14
10 k	17	14

such a waiting period ought to be sufficient for the greater part of the improvement to occur.

Day to day variations in middle ear pressure might well be responsible for some of the changes in acuity which are found. Close & Ireland (1961) have shown that a negative middle ear pressure of 10 inches of water produces up to 15 dB loss of sensitivity in threshold at 500 c/s and less at other frequencies. This suggests that variation in pressure difference across the tympanic membrane of the order of one inch of water could cause changes in acuity of one or two dB. In experiments on the acoustic reflex with normal ears day to day changes of this order in middle ear pressure were found to be not uncommon although we did not have the opportunity to measure thresholds on these occasions (McRobert & Bryan, 1967). This suggests that a small improvement in accuracy in threshold measurement could be obtained if audiograms were determined only after equalisation of middle and outer ear pressures. Whilst this would be possible in MAP measurements it would clearly not be so with MAF determinations.

The variance term (σ_p^2) has an entirely physical cause, that is, the uncertainty introduced due to the removal and replacement of the subject in the sound field. As is shown in Table 2 for MAF thresholds, the term (σ_p^2) is negligible compared with the other components of variance in eq. (1). This assumes careful measurement of the sound field from the

TABLE 2.

Frequency (c/s)	Total variance (σ_T^2)	Components of variance (dB²) Acuity (σ_a^2)	Sound field (σ_p^2)	Judgmt. & app. (σ_j^2)
100	3.5	2.2	0.0	1.3
500	5.3	2.7	0.8	3.3
1 k	6.5	2.9	1.4	2.2
3 k	4.4	9.6	0.8	3.2
6 k	14.0	10.6	3.7	7.1
10 k	14.3	6.8	0.3	7.2
Average	8.0	4.3	0.4	4.1

TABLE 3

Frequency (c/s)	Changes in acuity σa^2 (dB)	
	Naive	Practised
100	3.7	1.3
500	4.5	0.4
1 k	7.5	−1.5
3 k	1.7	−0.4
6 k	13.7	7.9
10 k	4.0	9.2
Av	5.8	2.8

loudspeaker before and after threshold measurement and that the sound field is uniform at the subject within some ±3 dB between 100 c/s and 10 kc/s.

Psychological causes

One psychological cause of some of the long term change of acuity (σa^2) would appear to be the degree of training the subject has had in making threshold judgements. Those subjects with no previous experience in audiometric testing gave in general greater values of (σa^2) than did those who had become highly trained as a result of taking part in scores of listening tests. This is shown in Table 3, the data being taken from unpublished work of the authors. Thorough training might therefore be expected to give an improvement of threshold variance of about 20–30% which would be useful for research purposes but not practical for routine audiometry.

The effect that day to day changes in the subject's criterion have upon the threshold are not known, but as this criterion would appear to be determined by so many factors such as alertness, confidence etc., it could well be responsible for a substantial part of (σa^2).

Residual variance

In their experiment Hempstock, Bryan & Webster (1966) found the residual variance [(σt^2) due to uncertainty of signal level and short term physiological changes in the ear and σj^2 due to short term changes of subject's criterion of what is his threshold] had a not insignificant average value of 4 (dB)². It is unfortunately not possible in conventional audiometry to separate this residual variance into its two components σt^2 and σj^2 although clearly the first term is due to physical and physiological factors whilst the second term is psychological in origin. We might suppose that σj^2 is the main part of this variance as it is found that changes in signal level from test to test are less than 1 dB. Also it is unlikely that conditions in the ear will change by more than 1 dB over a test period of an hour (Hempstock, Bryan & Webster, 1966).

Tinnitus

Tinnitus may well be responsible for some of the increase of both terms σ_a^2 and $(\sigma_f^2 + \beta^2)$ with frequency as indicated in Table 2. Subjects appear to have greater difficulty and uncertainty in detecting the signal as its frequency rises. It would not appear possible that the effects of tinnitus upon threshold (if it is genuine) could be eliminated.

From this examination of the components of variance it may be concluded that some factors such as previous noise exposure training, etc can be controlled and by this means threshold variance could be reduced by possibly up to a factor of two. However other factors such as tinnitus change of subject criterion etc. would appear to be beyond our control For greater improvements in accuracy it appears that it will be necessary to seek improved methods of measurement.

Signal detection theory

In order to obtain more precise thresholds a method is needed which will enable us to obtain independent estimates of the so far unresolved component of variance which arise in different parts of the hearing process.

In the foregoing section we have indicated that there are likely to be both physiological-cum physical and psychological causes for the variation in the threshold. The former would appear to affect the detectability of the signal in the ear itself and the latter determine the criterion taken up by the subject of exactly what is going to be called a threshold.

Clearly most hearing disorders and noise induced hearing loss affect only the detectability of the signal by the ear itself while it is not unreasonable to assume that the process of judgement of what constitutes a threshold occurs in the higher centres of the brain and is unaffected. Thus there would seem to be no point in measuring some quantity which will remain unaltered whether any hearing disorder is present or not particularly if by concentrating on the detectability of the signal alone we are able to make more precise measurements.

The general theory of signal detectability as presented by Peterson, Birdsall & Fox, in 1954 and applied to psychophysics for the first time by Tanner & Swets in the same year claims to be able to give independent estimates of the detectability of the signal and of the subject's criterion. If this theory could be successfully applied to audiometry then more precise thresholds might be possible It remains to briefly describe the threshold model the theory proposes, and to consider how the theory could be adapted to audiometry

Signal detection model of threshold

In the theory the process of signal detection in noise is considered to be a choice between two Gaussian variables. One having a mean arbitrarily taken as equal to zero is associated with noise alone the other having a

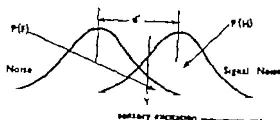


FIG. 1 Fo an ideal observer (Peterson *et al* 1954) $d' = (2E/No)^{\frac{1}{2}}$ and Y is criterion operating point on sensory excitation scale where

E is the signal energy; No is the noise power/unit bandwidth

This Figure hows the n ise di t ibution and its displacement to the right with increa ing sensory excitation when the signal is present as well as the noise Y is the subject s operating point or criteri n cut-off and is determined by the subject s hit rate *P(H)* and false alarm rate *P(F)* If the excitation falls below Y then the subject indicates n ignal and if it falls above Y he indicates the presence f the signal. In the simplest form of signal d tecti n experiment—that of Free Choice—the subject is asked t say whether he pref rs the deci ion that the ignal is present or abse t in interval of whit noise About one hundred presentations f intervals of noise with fixed ignal/noise ratio and *a pri ri* noise distribution re necessary t determine *P(H)* and *P(F)* and henc *d'* and som measure of Y with reasonabl accuracy

mean equal to the detectability *(d')* is associated with signal plus noise In the simplest detection experiment that of Free choice the observer decides which of the two alternatives was presented to him in a series of observation intervals. The decision for any given observation interval depends upon whether or not the observation exceeds a criterion value (Y) on some scale of sensory excitation (see Fig 1) and this criterion depends upon the observer s detection goal and upon the information he has about the relevant parameters of the detection situation The term *d'* is defined as the difference of the means of the two gaussian density functions expressed in terms of their standard deviations and is therefore a dimensionless quantity

It has been shown experimentally for various types of stimuli that the detectability *d'* changes markedly with the signal/noise ratio and is not influenced by changes in instruction to the subject concerning his judgement criterion Measures of the cut-off criterion of the subject are affected by different instructions but are independent of the signal/noise ratio (Fairbanks, House & Melrose 1956)

Here we have a technique for signal detection in noise which gives us independently a measure of the objective performance of the ear in determining the detectability of the signal *d'* and of the subject s criterion of judgement It is precisely these two measures it was suggested earlier which must be determined if more precise threshold measurements are to be made

In the next section we shall examine the problems which must be overcome if these signal detection procedures are to be applied successfully to routine audiometry

Problems of precision audiometry

The theory of signal detection is based upon the detection of signals in external white noise. The first difficulty to be overcome is in deciding whether such a model of threshold will be valid in the absence of external noise—the conditions under which conventional threshold measurements are made. The only noise present in the latter case is internal and arising presumably in the ear and the auditory pathways to the brain. It appears from the work of Eijkmann & Vendrick (1964) that the probable distribution of the internal noise can be considered as Gaussian and therefore it would appear to be valid to apply the model to quiet threshold measurements.

In the usual signal detection experiment it takes as long to obtain a single data point as to obtain complete audiogram for both ears using conventional audiometric techniques. There must therefore be a considerable peeding up of data collection in any form of signal detection audiometry. This will require (1) the use of some threshold seeking technique such as that used by Brown (1965) to determine the optimum signal level for which the detectability d′ would be most accurate and most sensitive t changes in the condition of the ear (2) the process of data collection nd calculation of threshold measures be automated.

Finally it will be necessary to compare and correlate the measures of thresholds btained by the signal detection technique with those given by con entional audiometry.

ZUSAMMENFASSUNG

Wiederholung unter Ohrschw llenva ianz kann man ich als aus Variablen bestehend vorst llen, die entweder mit dem Sinnessystem oder mit dem Kriterium erbunden sind, wonach d Versuchsperson ihre Schwelle misst. Die konventionelle Audiometrie kann nicht die Wirkungen dieser verschied en Qu llen auseinanderhalten. Die Theorie der Zeichenermittlung scheint, wenn m n sie bei der I \ bophysik nwendet, eine Methode zu gestatten die es erlaubt, diese verschiedenen Wirkungen getrennt zu messen. Die V rianz jeder dieser V riablen sollte in ll ht auf normale Ohren kleiner als die Varianz kon entioneller Schwellenerm ttlungen sein. Daher konnte das Verhalten normaler Ohren präziser angegeben werden, und deshalb sollten Abw ichungen von der Normalität schn ller entdeckt werden al bisher. E werden die Probleme erörtert, di gelöst werden müssen wen man die Theorie der Zei henermittlung der täglichen Praxi der Audiometri erfolgreich pa sen will.

REFERENCES

Arrowsmith, G. R. C., nd Lowe, P. 1963 a. A preliminary tudy f the effect f earphone position on the reliability of repeated uditory threshold determinati ns. J. L Audiol. 4 161

Arrowsmith, G. R. C., Lowe, P. and W..., J. G. 1963. A circuma l earphone for use i M.A.P. determinations. *Proc. 5th I.C.A.* Liege 1 J 32.

Brown, A. E., 1963. Measurement of uditory thresholds. *J. Acoust. Soc. Am.* 35 84

Bryan M E., Parbrook, H D and Tempest W 1965 a A not on quiet threshold shift in the absence of noise J S und Vib 2 147
— 1965 b Variation of quiet thresholds with low level noise exposure Proc 5th I.C.A., Liege 1 B 32.
Close, P and Ireland R G 1961 Alterations in pure tone threshold following changes in both absolute and differential pressures upon the ear J Audiol Res 1 134
Eliemann E., and Vendrick, A J H 1964: Signal diction and recognition by human observers (Ed Swets, J A) Ch 18, J hn Wiley New York
Fairbanks, G Hol se, A S and Melrose, T 1956 Audit ry d tection of presence and absence of signal in noise J Acou t Soc Amer 28 614
Hempstock, T H Bryan M E., and Webster, J B C., 1966 Free field threshold variance J S und Vib 4 33
McRobert H and Bryan M E.: In preparation
Peterso W W Birdsall, T G., and Fox, W C., 1954: The theory of signal detectability In t Radio Engrs Trans. Inform Theory 4 171
Robinson, D W 1960 V riability in the realization of the audiometric z ro Ann. Occup. Hyg 2 107
Tanner, W P Jn and Swets, J A 1954 A decision making theory of visual detection. Phychol R v 61 401

D pt Elect ical Engineering University of Salford S1lf rd Lancs England

Rec ived July 30 1966

Acta oto-laryngologica 64 213-218 (1967)

LYMPHOEPITHELIOMA OF THE NASOPHARYNX

Cervical Adenopathy Diagnosed Late

G. EVERBERG K. E. SJÖLIN and I ØRNTOFT
Copenhagen Denmark

From the E N T Department (Head G Eve berg) and the Instit te of Pathology (Head K E Sjölin) Sundby Hospital, C penhagen

A case of nasopharyngeal lymphoepithelioma in a 16-yea -old boy is reported. Ten months elapsed f om the first manifestation swelling of the cervical lymph nodes, until the diagnosis was made The first biopsy from the nasopharynx was negative Four bi psies from the cervical ode were negative All th findings indicated infection, until the second biopsy from the nasopharynx disclosed the true nature of the disease

Th 4 egati cervical-nod biopsies are remarkable The most likely xplan tion is lymphoid overgrowth of the ca cer tissu The neces ity of continuing to remo biopsies from swollen cervical nodes as well a from the nasopharynx is emphasi ed, all the more so as the disease is amenable t radiotherapy f t is diagnosed in time

Lymphoepithelioma of the nasopharynx is a rare type of tumour Among Godtfredsen s material (1944) comprising 4 4 malignant tumours of the nasoph rynx lymphoepithelioma made up only 10.6% Since however thi tumour is particularly radiosensitive it is more amenable than other maligna t tumours of the nasopharynx to curative treatment, *if it is diagnosed in time*

This i absolutely decisi e In Godtfredsen s series the average period from th first sign until the tumour was diagnosed was 11 months. In Sm th & Reeves series (1939) of 48 cases thi interval averaged 8 months, ranging from 1 month t 3 years. The diagnosis ha usually been made on the ba i of biopsy from cervical nodes to which the tumour has m ta t sized

Below f llows a case hi tory illustrating how difficult it may be to arrive at the diagnosi of lymphoepithelioma.

Th patient was a boy f 16, who had previously been in good health part fr m bronchiti at the age of 7 and 2 attacks of tonsillitis, most recently 6 month before the present disease There was no family history nd th patient had had all the conventional vaccinations except for BCG.

Three weeks before being admitted to the E.N.T. Department, the patient began to notice a swelling below the right angle of the jaw. This swelling gradually grew to the size of a closed fist. The only other sign was elevation of temperature (37.7–8 to 38.3–8 C).

Objective findings on admission: Temperature 38.6. General health unaffected. On the right side of the neck there was a firm, almost smooth swelling with a trace of grooves. The swelling was the size of a closed fist and it was tender to pressure. It did not adhere to the skin or underlying structures and reached to a site 2 finger breadths from the clavicle. The skin was normal. No nodal enlargement elsewhere. Spleen impalpable. Pharynx normal.

Nasopharynx: Medially to the orifice of the right Eustachian tube there was a moderate lobulated mucosal thickening suggestive of adenoid vegetations. Owing to a suspicion of tumour, especially as the E.S.R. was 69 mm, a biopsy specimen was removed from this site under visual control using a Well forceps. The histological report was: "Adenoid vegetations. No signs of specific inflammation or malignancy."

Among other findings there is reason to mention: Hb 97%, R.B.C. 4.93 mill., colour index 0.95, W.B.C. 13 080, differential count: no abnormality. The Bunnell reaction was negative. X-rays of the lungs and cervical spine showed no abnormalities.

The disease was interpreted as an infection, and the patient was given sodium penicillin 1 mill. units twice daily i.m., for three weeks. However, as the cervical swelling remained unchanged the first nodal biopsy was removed 18 days after admission, which was 6 weeks after the first clinical manifestation. The biopsy specimen showed a smooth, tense capsule without neoplastic characteristics. The tissue looked like lymph node tissue and the histological report said: "Lymph node with fibrosis and non-specific inflammation." Culture for T.B. was negative.

During the penicillin medication the temperature as well as the erythrocyte sedimentation rate fell, and as the patient was feeling well in spite of the unchanged swelling, he was discharged. The E.S.R. decreased during the subsequent period to 27 mm.

Six weeks later the patient was re-admitted in a state of hyperpyrexia with severe pain in the neck. The swelling on the neck remained unchanged. The pharynx was reddened. The E.S.R. had risen to 103 mm and the W.B.C. to 18 320, now with some shift to the left. Hb 96%. On the assumption of an adenophlegmon with profound suppuration, the most prominent part of the swelling was incised under antibiotic cover (penicillin and streptomycin). This revealed 2 large lymph nodes. The capsules were opened but contained no pus. The tissue was white, firm, and uniform. Probing into the depth disclosed no pus. Histological report: "Lymph node with fibrosis and eosinophilia." The patient had now been ill for 3 months.

After consultation with the internists, a comprehensive haematological

and serological investigation was done. This included inter alia test for L.E. cells, cold agglutinin titre toxoplasmosis reaction, Bunnel Widal, and W.R., all of which were negative, and finally serum electrophoresis which showing elevated α and γ-globulin values, indicated infection Control X-rays were obtained of the lungs, with normal findings, and the sternal bone marrow showed Hyperplastic bone marrow with increased myelopoiesis, without evidence of systemic malignancy specific inflammation, or tumour formation"

Shortly after the patient voided large quantities of purulent secretion by mouth, without any perforation or oozing of pus being demonstrable in the hypopharynx. Thereafter he felt better

The third nodal biopsy was obtained 3½ months after the onset of the disease in an attempt to demonstrate bacteria, Toxoplasma, Coxsackie, polio or ECHO virus, but with negative results. The histological examination afforded no new data

The fourth, and last, incision of the swelling was done after 4 months' illness, when fluctuation had appeared superiorly on the neck. The swelling appeared to be multilocular A thin fluid was evacuated. The incision had led right to the larynx and the uppermost tracheal ring We abstained from total excision because of the large size of the swelling and its great tent A piece of capsular tissue was sent for microscopic examination, nd the report was "Chronic non-specific inflammation.

In nticipation of a fa ourable effect of X ray therapy upon this "non-specific inflammation irradiation f the right side of the neck was started, more than 4 months after the onset of the disease The total dosage was 150 r 3, and this resulted in considerable subjective as well as objective improvement The E.S.R. fell from 123 to 52 mm, the Hb level nd white cell count returned to normal, and the patient was discharged

At follow up 7 weeks later he had no cervical complaints, and only an nfiltrated rea remained on the right. The patient was fit apart from a c mmon ld and a stuffy feeling in the right ear Unfortunately the latter was interpreted as part f the catarrhal condition as the mucous membran of the rhinopharynx exhibited only slight reddening and especilly as th E.S.R. w 13 mm and the Hb 112%

A few month later he still had impaired hearing in the right ear and the drum w greatly retracted. Indirect nasopharyngoscopy now revealed The right ide f the fornix wa prominent infero-medially cov red with ppa ently normal mucosa A biopsy pecimen was removed from the rea Now 10 months fter the onset f the disease we g t the surprising d gn si "M lignant n plastic tumour (lymphoepithelioma or transl t l-cell carcin ma)



Fig 1 Lymphoepith li ma Th pale tum ur cell are rra g d In broad Irregular f selcles l t rmingled with l mphocytes Hæm t x lin-eo l × 40

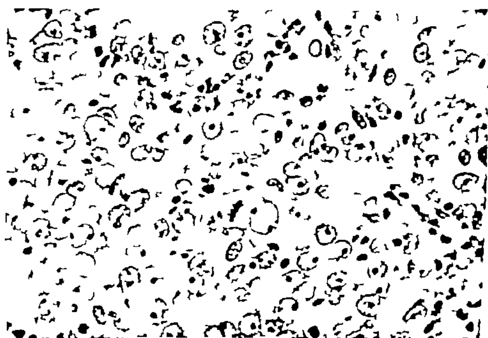


Fig 2 Lymphoepithel m The tum cell re large d pal w th d f t o l ll es Th v l nuel l re esleal r Th hrom ti co t t l poor O m re nucleoli re oft present in th n l l A mitosl seen Between the t m cell f w lymphocytes are dl persed Hæmat ll -eos n 400

DISCUSSION

The symptoms and signs of malignant nasopharyngeal tumours are generally a follows Nodal swelling on the neck, hearing impairment be cause of occlusion of the Eustachian tube, nasal complaints (stenosis, discharge epistaxis) neuro-ophthalmological symptoms, such as trige minal neuralgia, lesion of the optic nerve (2nd, 3rd 4th 6th cranial nerves) and invasion into the base of the skull with destruction The most common initial sign of lymphoepithelioma is swelling of the cervical lymph nodes and tubal occlusion, as in our patient. It is characteristic (Smith & Reeves, 1939) that this tumour invades beneath the nasopharyngeal mu cosa without ulcerating it.

The treatment i radiological a the lymphoepithelioma is extremely radiosensitive Our patient improved rapidly on X ray therapy in the Radium Centre The nodal swelling as well as the nasopharyngeal tumour disappeared entirely The patient is completely fit at follow-up now 6 years after the onset of the disease He had received a total of 6000 r distributed on 2 periods at an interval of 6 months

The prognosis i fairly good, if the treatment is instituted early The 5-year survival ranges from 22–42% and the degree of malignancy is greatest below the age of 20 Those who manage the first three years without recurrence will also, according to Smith & Reeves, be found among the seven survivors. Thus, our patient ought to have a favourable prognosis.

A remarkable feature is the very long period from the onset of the disease until the diagnosis was made ix 10 months As i apparent from the literature this i the rule rather than the exception As already mentioned, the average interval in Erik Godtfredsen's material of malignant nasopharyngeal tumours was 11 months In Smith & Reeves study on nasopharyngeal lymphoepitheliomas, the corresponding interval was 8 months from the initial sign until the diagnosis. Some patients have had number of biopsies from the nasopharynx until any diagnosis could be established In several cases, positive findings have been made from cervical nodes before the nasopharyngeal disease has been recognized

In our patient it was the other way round Four biopsies from the cervical nodes were negative and the diagnosis was not made until the second nasopharyngeal biopsy In three of Godtfredsen 454 patients with reticulum-cell car in was the report on the nodal biopsies had been similar chronic non-specific lymphadenitis, in spite of the fact that the nodal swelling had persisted for 6–12 months, and another 6–11 months elapsed until positive report was made This means 2 years, 18 months, and 10 months respectively from the onset of nodal swelling

There is only one source in the literature which gives any explanation of why the biopsy findings from the nasopharynx are so often negative even when there is strong suspicion of tumour in this region Morlin & Blady (1940) believe that the tumour is hidden in hyperplastic lymphoid

tissue or in granulation tissue since *there is no other site of the upper respiratory tract or of the alimentary tract in which cancer is so apt to be overgrown by this tissue* These authors do not mention the negative cervicalnode biopsies, but it seems reasonable to expect corresponding reactive changes in the regional lymph nodes as in the lymphoid tissue of the nasopharynx, caused by the nasopharyngeal tumour before metastasization sets in

Nodal swelling on the neck is in most cases a sign of a benign disease of the upper airways or the digestive tract but in a few cases it may be the first sign of a malignant disease Nasopharyngeal cancer in particular is difficult to diagnose Therefore in the event of long lasting nodal swelling on the neck no reliance should be placed on one negative biopsy The nodal swelling should be suspected of being secondary to a malignant nasopharyngeal tumour The biopsies—from the cervical nodes as well as from the nasopharynx—should therefore be repeated as long as the patient has inexplicable symptoms.

ZUSAMMENFASSUNG

Ein Fall von Lymphoepithelioma Rhinopharyngis bei einem 10jährigen Mann wird erwähnt 10 Monate verliefen vom ersten Symptom (Drüsenanschwellung am Hals) bevor die *Diagnose erkannt wurde* Die *erste* Rhinopharynx Biopsie war negativ 4 Halsdrüsen Biopsien waren negativ Alle Untersuchungen wiesen auf eine Infektion hin bis zur letzten Biopsie (der zweiten Rhinopharynx Biopsie) die die wahre Natur des *Leidens enthüllte* Bemerkenswert sind die vier negativen Halsdrüsen Biopsien Dieses erklärt sich wahrscheinlich durch das lymphoide Überwachsen des Karcinom Gewebes. Die Notwendigkeit wieder holter Biopsien von geschwollenen Halsdrüsen sowie von Nasopharynx wird hervorgehoben um so mehr als das Leiden kurativer Strahlentherapie zugänglich ist wenn es zeitig erkannt wird

REFERENCES

GODTFREDSEN, E., 1944–45 Ophthalmologic [illegible] d [illegible] urol gic [illegible] mpt m [illegible] t mal gnant [illegible] d a ph ryngeal tum ars *Acta Otolaryng (Stockh)* Suppl 59

M[illegible] H E., and B[illegible] J V 1940 Cancer of th[illegible] n soph[illegible] *Arch Otolaryng (Chic)* 33 602

SMITH C A and BARNES, R J 19[illegible]9 Lymphoepithelioma of th[illegible] ph[illegible]n[illegible] *North Carolina Med J* 20 213

G Everberg M.D the E N T Department Sundby Hospital Copenhagen Denmark

Received December 21 1966

Acta oto-laryngologica 64 219–225 (1967)

HEREDITARY NERVE DEAFNESS ASSOCIATED WITH DIABETES

S. Hognestad
Molde, Norway

From the Ear, Nose and Throat Department (Head: Ivar Brekke)
Fylkessjukehuset, Molde

A family in which hereditary nerve deafness occurred together with diabetes is reported. The inheritance pattern is dominant and the hearing loss is of a slowly progressing type with a sloping audiometric curve. The diabetes seem to make the hearing loss worse, but the genetical relation between the diabetes and the hereditary nerve deafness has not been established. There are also in the family other developmental anomalies, and the possibility that this might be part of a full syndrome is mentioned.

INTRODUCTION

This is a report on a family in which hereditary nerve deafness in some cases occurred together with diabetes. There were also in the family cases of other developmental anomalies. The aim of the investigation was: 1. To clarify the mode of inheritance. 2. To try to find out if there was any connection between the diabetes and the deafness.

According to Johnsen (1952) and Altmann (1964) hereditary nerve deafness may be classified in two main groups.

1. Hereditary malformation of the inner ear
2. Hereditary degeneration of the inner ear

In the first group, deafness is *present at birth. In most cases the speech* development is disturbed and many of these patients are deaf mute.

In the second group hearing is normal at birth, but later it decreases, in most cases during adolescence. The hearing impairment very seldom start before the age of six, or *after the age of 30–35. As a result,* speech development is normal or nearly normal.

In heriditary nerve deafness, the hearing impairment may be the only defect or it may occur together with other abnormalities such as pigment changes of the skin and retina (Woolf, *Dolowitz & Aldous,* 1965), Waardenburg's syndrome (Waardenburg, 1937; Aa ed, 1962; Thorkilgaard, 1962) or in combination with renal anomalies or nephritis (Johnson & Hagan, 1965; Beickert, 1966).

The mode of inheritance in hereditary nerve deafness is dominant, recessive or recessive sex linked (Cawthorne & Hinchcliffe 1957) It was formerly believed that deaf mutism was transmitted as a recessive trait, while partial nerve deafness followed dominant inheritance Johnsen found, however in 1952 that there were cases in which incomplete loss of hearing was transmitted as a recessive trait Instead of speaking of partial and complete loss of hearing he classifies hereditary nerve deafness in a progressive type with a dominant inheritance and a non progressive type which is recessive

The audiometric pattern in progressive hereditary nerve deafness is, in the earlier stages that of a high tone loss later progressing to the middle and low frequencies as shown by Dolowitz & Stephens, (1961) and Huizing Bolhuis & Odenthal (1966) Cawthorne & Hinchcliffe in 1957 also find that hereditary degenerative nerve deafness transmitted as a dominant trait has a flat or sloping audiometric curve It thus seems to be justifiable to conclude that most investigators find that in hereditary degeneration of the inner ear the transmission is by a dominant gene with a sloping or flat audiometric curve beginning as a high tone loss of hearing There are however exceptions to this rule both concerning the audiometric curve (Martensson 1960) and probably also concerning the inheritance pattern (Cawthorne & Hinchcliffe 1957)

Information concerning the inner ear changes in hereditary nerve deafness is scarce Altmann (1950 1964) in most cases found changes in the organ of Corti and stria vascularis, more seldom in the cochlear nerve fibres and spiral ganglion cells

In diabetic patients, most investigators find a slowly progressing hearing impairment affecting the higher frequencies first Jørgensen in 1961 in 69 diabetic subjects found 28 cases with hearing impairment presumed to be caused by diabetes In the age group under 30 years of age, the loss of hearing was slight and it was most often present in older diabetics. The hearing loss was bilateral affecting all frequencies with a preponderance for the higher tones

Jørgensen (1961) studied the inner ear in diabetics and he found changes in the capillaries in stria vascularis It thus seems to be difficult both by audiometric studies and by histological investigations, to draw a clear distinction between the hearing loss in diabetes and that in progressive hereditary nerve deafness But one may presume that if the two diseases occur together the loss of hearing will increase

INVESTIGATION

The family in the present investigation consists of 45 members in three generations (Fig 1) The original parents had 12 children and it is this second generation that has been most carefully studied All the living members in this generation have been studied audiometrically by pure

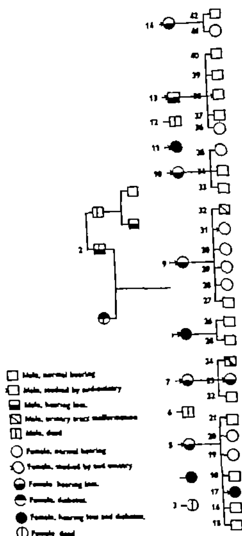


Fig. 1 Family pedigree

tone audiometry In the third generation 4 members have been studied by audiometry two of which were found to be hard of hearing. Except for these two members there is no information of hearing loss in the third generation, but most of them are too young to be expected to present the trait. A total of 12 persons have been studied by audiometry Some of the audiograms are shown in Fig 2 A few points of interest in some of the family members are as follows

1 Father of the family Hard of hearing from his thirties. He did not use hearing aid. A son of his brother is also hard of hearing.

2. Mother of the family She had diabetes and reduced hearing in her late years.

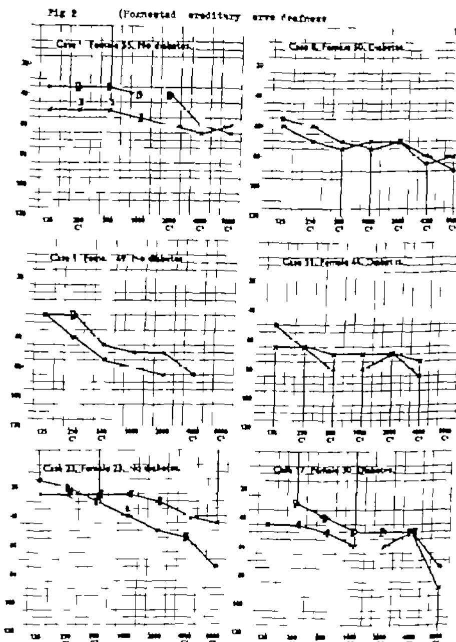


FIG 2 Audi gram from som of the family members, all f mal Case numbe ind cates numbe in pedigree Number foll wi g "Femal indicates ge at the tim f i vestiga ti n Where th bon conducti n igns re omitted th bo conducti n i bel w the c pa ity f the udi meter

3 The oldest member of the second generation Born 1907 Died 1910 She was a cripple but it is not known what the defect consisted of

4 Born 1909 died 1931 of diabetes. She had reduced hearing, but did not use an aid

5 Born 1910 She has reduced hearing but is not using an aid and has not *diabetes*

6 Born 1911 Male died 1917 of pneumonia No diabetes and normal *hearing*

7 Born 1913 She has not noticed any hearing impairment herself but a pure tone audiogram taken in her home showed a hearing loss for the higher frequencies. No diabetes.

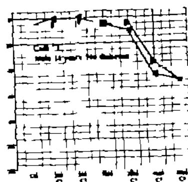


Fig. 3 Audiogram of the only male member alive in the second generation, showing high tone loss quite different from the hearing loss in the other family members. The hearing loss in this case most likely is due to noise exposure

8 Born 1915. She is under treatment for diabetes, has pronounced loss of hearing, and is using an aid.

9 Born 1916. Died 1965. She had not diabetes, but severe hearing impairment and was using an aid.

10 Born 1919 She has a double uvula, and a moderate hearing loss, is not using an aid. No diabetes.

11 Born 1921 She has diabetes, and a severe hearing loss. She had to start using a hearing aid after her diabetes became manifest.

12 Born 1924 Died 1941 He had a deformity of his legs and was oligofren. He was suffering of epileptic seizures. His hearing was probably normal, but he never developed intelligible speech

13. Born 1922. He has high tone hearing loss (Fig. 3) most likely due to exposure to noisy environment as he has been whaler

14 Born 1927 She has a moderate hearing loss, is not using an aid, and has not diabetes.

In the third generation there are positive findings in the following members

17 Born 1933, the daughter of no 5 She had to start on insulin due to diabetes 1961 She had then for some years noticed hearing impairment, but the few months preceding the detection of her diabetes, she had noticed a pronounced hearing decrease and she had to start using a hearing aid.

23 Born 1943 She is the daughter of no. 7 and has perceptive high tone loss.

1 The son of no. 7 He died few weeks old, and till then he had only passed few drops of urine

32 The son of no. 9 There is no information as to reduced hearing, but he has been operated on because of uretero-vesical stenosis.

DISCUSSION

In this family there is no information as to the parents being related. It is known that the mother of the family had diabetes and reduced hearing in her late years, but one does not know if the loss of hearing in

her case was an ordinary presbyacusis or whether it was due to diabetes. The father however had already reduced hearing in his thirties, and the gene leading to hearing impairment probably comes from him

The mode of inheritance is obviously dominant (Fig. 1) as there is a manifestation of more than 50% in the second generation The great prevalence of cases of deafness in women might point to a dominant sex *determined inheritance with the gene for hearing loss in the x-chromosome* At the present stage this cannot be established with certainly The *hearing loss is of the progressive type* so one can *classify this as a* progressive perceptive deafness with dominant inheritance.

The next characteristic feature is the large number of diabetics in this family Nearly 50% of the patients with reduced hearing have also diabetes. Besides, there is diabetes in three consecutive generations. As diabetes is usually considered to be transmitted recessively it might look as if the gene of hearing loss could lead to an increased penetration of the diabetogenic gene also

The third point of interest is the influence of diabetes on hereditary nerve deafness In this material it looks as if diabetes worsens the existing deafness, as we know that hearing became worse in relation to manifestation of diabetes (cases 11 and 17) At the same time it seems that patients with diabetes are on the whole more seriously *affected* (Fig 2) and all diabetics needed a hearing aid while only one of the others was using one

The fourth point of interest concerning this family is the existence of double uvula and congenital urinary tract anomalies in some members *(cases 10 24 and 32) In no. 24 one has reason to* believe that the cause of death was maldevelopment of the urinary tracts In no 32 we know that *the anomaly consisted of a uretero-vesical stenosis*

Braun & Bayer described in 1962 a family in which reduced hearing *occurred together with urinary tract anomalies congenital* double uvula and anomalies of the thumbs and big toes This might make us believe *that there is a syndrome in which hearing loss may occur* together with urinary tract anomalies, double uvula digital malformation and perhaps *diabetes In any case it seems to be something more than* a coincidence that one finds in this family too anomalies in the urinary tracts and *uvula*

ZUSAMMENFASSUNG

Eine Familie in welcher erbliche Innenohrschwerhörigkeit und Diabetes auftraten wurde untersucht Der Gehörverlust ist von einer langsam fortschreitenden Art mit grösstem *Verlust der höheren Frequenzen Es wird angenommen* dass sich der Gehörverlust durch gleichzeitigen Diabetes verschlimmert aber der erbliche Zusammenhang *zwischen Diabetes und Gehörverlust lässt sich nicht* erklären In der Familie kamen auch andere Entwicklungsstörungen in der Form

einer doppelten Uvula und Malformation on Urinwegen vor und di Möglichkeit besteht dass dieses ein T il von einem Syndrom ist.

REFERENCES

Arnvig, H 1962 Waardenburg' syndrome *Act Otolaryng* (Stockh.) *50* 622.

Altma F 1950 Histologic picture of inherited nerv deaf ess i Ma and nimals. Arch Otolaryng (Chic.) *51* 852.

— 1961 The i ner I genetically determined deafness. *Act Otolaryng* (Stockh.) S ppl *187*

Beckatat P 1964 I nenohrschwerhörigkeit bei erblicher Nephropathie *Z Laryng Rhinol Otol 43,* 224

Bra F C., nd Baten, J F 1962 F milial nephrosis ass. with deafness nd cong urinary tract nom. I siblings. *J Pediat 60* 32.

Cawthorne, T E., and Hi cncliffe, R 1957 F milial perceptiv deafness. *P act Oto-rhinolaryng* (Basel) *19* 32.

Dolowitz, D A nd Stephens, F E. 1961 Hereditary nerv deafness. *Ann Otol 70* 851

H erive, E. K., Bolmi n, A. E., and Onerymal, D W 1964 Studies on progressi hered t ry nerv deafnes i f mily of 335 members. *Act Otolaryng* (Stockh.) *61* 33 nd 161

Johnsen, S., 1952 Heredity f percepti deaf ess. *Act Otol ryng* (Stockh.) *42* 539

Jo son, W nd Hans P J 1965 Heredit ry nephropathy and loss f hearing. *A ch. Otolaryng* (Chic) *82* 166

Jorge we, M B., 1961 Inner ea in diabetes mell tus. *Arch Otol ryng* (Chic.) *74* 373.

Jorgensen, M B., and Buch, N 1961 Studies of inner ear function nd cra i l nerves i diabetes. *Act Otolaryng* (Stockh) *64* 579

Stenz son, B., 1960 Domi nt hereditary nerv deafness *A t Otol ryng* (Stockh.) *52*, 279

Thorarinsson, O 1962 Waardenburgs yndrome i f ther nd da ghter *Act Ophthal* Kbh. *40* 580

W aardenburg, P J 1957 Hyperplasia interocularis cum dystopia lateroversa canthi medialis, blepharophimosis, dyschromi iridorum sea et dysplasia dilitva. *Act Ophthal* (Kbh.) 35 311

Woolf C. M Dolowitz, D A. nd Aldous, H. E., 1965 Cong ti l deafnes associated ith piebaldness. *A ch Otolaryng* (Chic.) *82* 244

E.N.T Department
Fylkessjuk h set Mold Norway

Received December 9 1966

Acta oto-laryngologica 64 226–234 (1967)

DIE TRÄGHEITSKRÄFTE IN EINEM BEWEGTEN BOGENGANGSMODELL ALS FUNKTION DER TORUSKOORDINATEN UND DER ZEIT

R GROHMANN
Göttingen Deutschland

Aus der Universitäts Hals Nasen-Ohrenklinik (Direktor Prof Dr A Miehlke), Göttingen

Es werden die Grundgleichungen angegeben mit denen die Geschwindigkeit der Flüssigkeitsbewegung und der Druck in einem bewegten Bogengangsmodell als Funktion der Toruskoordinaten und der Zeit berechnet werden können Die Eigenschaften und Besonderheiten dieses Ringmodells werden in bezug auf das menschliche Bogengangssystem diskutiert. Aus der Lage und Bewegung des Ringes im Raum folgt die Beschleunigung die wegen der Massenträgheit an der Ringflüssigkeit angreift und in die Grundgleichungen einzusetzen ist Sämtliche Kräfte die keine Verschiebung der Flüssigkeit im Bogengangsmodell verursachen werden bereits zu einem Partialdruck integriert Der Teilbeschleunigungsvektor der die Strömung bewirkt wird in Komponenten relativ zu einem ringfesten räumlichen Koordinatensystem zerlegt. Diese Komponenten und der Partialdruck enthalten nur bekannte Parameter wie die Abmessungen des Bogengangsmodells in Form der Reynolds-Zahlen die Dichte und Zähigkeit der Flüssigkeit die Winkelgeschwindigkeiten und beschleunigung des Ringes sowie die zwischen den Vektoren bestehenden Richtungscosinus und Entfernungen. Die Variation der Vektorrichtungen ermöglicht es später einige im Hinblick auf die menschlichen Bogengänge besonders interessante Lagemöglichkeiten des Ringes zu verifizieren Die Gravitation die keine Strömung in den Bogengängen erzeugt, bleibt unberücksichtigt Die Komponenten des Beschleunigungsvektors sind ebenso wie die zu berechnende Geschwindigkeit der Strömung und der Druck Funktionen der Raum Zeit Koordinaten

Für Kopfbewegungen in einem rotierenden System denen bei der Steuerung eines Flugzeuges oder eines Raumschiffes besondere Bedeutung zukommt haben schon Fischer (1928) Schubert (1934) Frenzel (1936/37, 1961) und Schindl (1957) die Richtungen der in den menschlichen Bogengängen einsetzenden Coriolisströmungen angegeben Die Kräfte die diese Strömungen verursachen wurden von Guedry u Montague (1961) in sehr vereinfachter Form mitgeteilt Das Ziel der vorliegenden Arbeit soll die Darstellung jener Trägheitskräfte in allgemeiner Form sein die in einem beliebigen Punkt der Flüssigkeit eines ringförmigen Bogengangsmodells

angreifen und in die Strömungsgrundgleichungen eingesetzt, später auch die Geschwindigkeit und Richtung der Flüssigkeitsbewegung im Modell zu berechnen gestatten. Unter spezielleren Annahmen als hier wurde in zwei früheren Veröffentlichungen (Grohmann 1962 1963) nur die tangentiale Trägheitskraft in einem Ringmodell abgeleitet und übersichtlich dargestellt. Diese zur ringförmig geschlossenen Zylinderachse des Bogengangsmodells parallele bzw. auf einer beliebigen Ringquerschnittsebene senkrecht stehende Komponente der Trägheitskräfte reicht jedoch nicht aus um die Flüssigkeitsströmung zu berechnen. Hierzu sind auch die in radialer und azimutaler Richtung eines Ringquerschnittes wirkenden Komponenten erforderlich. Auf eine mathematisch geschlossene Ableitung der Trägheitskräfte kann verzichtet werden weil ich mich im folgenden auf eine Mitteilung (Grohmann 1966) beziehen werde in der dies bereits geschehen ist. In der vorliegenden Arbeit werden die Ergebnisse lediglich so dargestellt, dass sie ein den Verhältnissen des menschlichen Bogengangssystems angepasstes Ringmodell hinreichend genau beschreiben. Die an der Flüssigkeit eines einfachen Bogengangsmodells angreifenden Trägheitskräfte der Druck in ihr und die Geschwindigkeit der Flüssigkeitsströmung sind Funktionen der Toruskoordinaten und der Zeit. Trägheitskräfte Druck und Geschwindigkeit können aus den Abmessungen des ringförmigen Modells der Dichte und Zähigkeit der Ringflüssigkeit sowie einiger im Experiment messbarer Parameter wie der Lage des Ringes seiner Winkelgeschwindigkeit und Beschleunigung im Raum, berechnet werden. Von den Grundgleichungen für eine räumliche Flüssigkeitsströmung ausgehend, ergibt sich von selbst die Notwendigkeit, erst die Trägheitskräfte herzuleiten welche die Bewegung der Flüssigkeit im Ring verursachen. Die Geschwindigkeit dieser Bewegung soll aber erst in weiteren Mitteilungen für speziell interessierende Fälle angegeben werden.

1 *Die Grundgleichungen zur Berechnung der Strömungs- und Druckverhältnisse in einem bewegten Bogengangsmodell*

Zur Beschreibung einer räumlichen Flüssigkeitsströmung muss die aus dem Energieerhaltungssatz der Mechanik folgende Navier-Stokessche Differentialgleichung in ihrer allgemeinen Form benutzt werden. Wird die Zeit [illegible] Differentiationsgrösse mit t bezeichnet und ist $\mathfrak{B}$ der an einem beliebigen Punkt der Flüssigkeit infolge ihrer Trägheit angreifende Beschleunigungsvektor so hat sie in kartesischen Koordinaten die Gestalt

$$\frac{\partial \mathfrak{v}}{\partial t} + (\mathfrak{v}\,\mathrm{grad})\,\mathfrak{v} + \nu\,\mathrm{rot}\,\mathrm{rot}\,\mathfrak{v} + \frac{1}{\rho}\,\mathrm{grad}\,p = \mathfrak{B}$$

Die Stoffkonstanten ν und ρ sind die als bekannt anzusehende kinematische Zähigkeit und Dichte der als inkompressibel vorauszusetzenden Flüssigkeit. Der Geschwindigkeitsvektor $\mathfrak{v}$ und der Druck p sind zu berechnen. Mit Hilfe des Ringquerschnittradius a und der Konstanten [illegible] ρ werden die folgenden dimensionslosen Grössen eingeführt.

$$\left.\begin{aligned} \mathfrak{v} &= \frac{a}{r}\bar{\mathfrak{v}} \qquad & p+q &= \frac{a^2}{\varrho r^2}\bar{p} \\ t &= \frac{r}{a^2}\tau \qquad & \mathfrak{T}+\mathfrak{K} &= \frac{a^2}{r^3}\mathfrak{B} \end{aligned}\right\} \tag{1}$$

Dabei wurden der Druck p und der *Beschleunigungsvektor* $\mathfrak{B}$ *derart in zwei* Teile zerlegt, dass sämtliche Kräfte, die keine Bewegung der Flüssigkeit, sondern nur einen Druck in ihr verursachen, in dem Teilbeschleunigungsvektor $\mathfrak{K}$ *zusammengefasst werden können. Wegen der Beziehung*

$$\mathfrak{K} = a \operatorname{grad} q$$

lassen sich $\mathfrak{K}$ und q aus der Navier-Stokesschen *Differentialgleichung* eliminieren. Diese nimmt dann dimensionslos geschrieben die nachstehende Form an

$$\frac{\partial \mathfrak{v}}{\partial t} + a(\mathfrak{v} \cdot \operatorname{grad})\mathfrak{v} + a^2 \operatorname{rot}\operatorname{rot}\mathfrak{v} + a \operatorname{grad} p = \mathfrak{T} \tag{2}$$

Zu dieser Grundgleichung tritt noch als zweite die auf den Massenerhaltungssatz zurückgehende Kontinuitätsgleichung

$$\operatorname{div} \mathfrak{v} = 0 \tag{3}$$

Bei Kenntnis des Teilbeschleunigungsvektors $\mathfrak{T}$ können mit diesen beiden, noch sehr allgemeinen Gleichungen die Flüssigkeitsströmungen in einem Bogengangsmodell für alle uns interessierenden Fälle berechnet werden. Nachdem man in dimensionsloser Schreibweise den Geschwindigkeitsvektor $\mathfrak{v}$ der Flüssigkeitsbewegung und den Druck p aus den Differentialgleichungen (2, 3) als Funktion der Raum-Zeit-Koordinaten in hinreichender Näherung gefunden hat, kann man diese Grössen in die Beziehungen (1) einsetzen. Es ergeben sich dann bei bekanntem *Druckanteil* q der experimentell nachprüfbare Geschwindigkeitsvektor $\bar{\mathfrak{v}}$ und der Druck $\bar{p}$. Der noch unbekannte Teilbeschleunigungsvektor $\mathfrak{T}$ und der Partialdruck q lassen sich *jedoch auf Parameter möglicher Exprimente zurückführen.*

2. *Eigenschaften und Besonderheiten eines einfachen Bogengangsmodells*

Während in den früheren Mitteilungen (Grohmann 1962, *1963*) zur Beschreibung des menschlichen Bogengangssystems ein aus drei Bogengangsringen bestehendes Modell benutzt wurde, dessen mögliche Kippbewegungen auf einen grössten Winkel von 90° beschränkt waren, genügt hier ein einzelner Hohlring mit kreisförmigem Querschnitt. Dieser soll aber beliebige Bewegungen im Raum ausführen bzw. die Lage des horizontalen oder auch vertikalen Bogenganges einnehmen können. Die Approximation des menschlichen endolymphatischen Bogengangssystems mittels eines einzelnen Hohlringes mit starren, undurchlässigen Wänden und überall konstantem kreisförmigen Querschnitt ist notwendig, wenn man für das physikalische Geschehen in einem der Bogengänge noch relativ einfache mathe-

matische Formulierungen anstrebt. Im menschlichen Ohr sind drei Bogengänge untereinander verbunden und besitzen nicht überall denselben Querschnitt. Das hier benutzte Modell berücksichtigt weder diese Verzweigungen und Querschnittsänderungen noch das Vorhandensein einer Cupula in jedem der drei Bogengänge. Um die Ergebnisse in möglichst allgemeiner Form schreiben zu können, werden für die Dichte ϱ und die kinematische Zähigkeit ν der Ringflüssigkeit sowie für den Querschnittsradius a und den Radius b des Ringes, welcher von dessen Mittelpunkt bis zu dem eines Ringquerschnittes reicht, noch nicht die für den menschlichen Bogengang angenommenen Zahlenwerte eingesetzt. Auf diese Weise wird gewährleistet, dass die abzuleitenden Gleichungen auch zur Beschreibung der Strömungsverhältnisse in physikalisch ähnlichen Ringmodellen herangezogen werden können. Es seien aber schon hier die angenäherten Werte der später zu benutzenden Abmessungen des Endolymphschlauches und der Stoffkonstanten der als inkompressibel anzusehenden Endolymphe angegeben.

$$\left.\begin{array}{ll} a = 0{,}02 \text{ cm} & b = 0{,}25 \text{ cm} \\ \varrho = 1 \text{ g cm}^{-3} & \nu = 0{,}01 \text{ cm}^2 \text{ s}^{-1} \end{array}\right\} \qquad (4)$$

Für die Dichte und kinematische Zähigkeit der Endolymphe wurden als Extrema die Werte, die für Wasser unter Normalbedingungen gelten, eingesetzt. Neben diesen vier Konstanten gehen noch die Orts- und Geschwindigkeitsparameter in den Beschleunigungsvektor $\mathfrak{T}$ und in den Partialdruck q ein.

3. Die Bewegung des Bogengangsmodells im Raum

Um aus der räumlichen Bewegung eines Bogenganges die Kräfte, die infolge der Massenträgheit an der Ringflüssigkeit angreifen, mathematisch möglichst einfach formulieren zu können, führt man zweckmässigerweise mehrere orthogonale Koordinatensysteme ein.

Das in den Abbildungen 1a und 1b eingezeichnete ringfeste Bezugssystem $\mathfrak{j}$, $\mathfrak{i}_r$, $\mathfrak{i}_\varphi$, dessen Ursprung in den Ringmittelpunkt $\mathfrak{M}$ gelegt wurde, rotiere mit konstantem Abstand d und zeitlich unveränderlicher Winkelgeschwindigkeit

$$\vec{u} = \dot{\psi}\,\vartheta$$

derart um den Vektor ϑ des ebenfalls im Raum beweglichen Dreibeins ϑ, ϑ_r, ϑ_φ, dass die Vektoren $\mathfrak{j}$ und ϑ_s stets senkrecht aufeinander stehen. Mit derselben konstanten Winkelgeschwindigkeit $\vec{u}$ bewegt sich dann der vom Koordinatenursprung O nach $\mathfrak{M}$ gerichtete Einheitsvektor $\mathfrak{r}$ um ϑ_r. Der zeitlich veränderliche Winkel φ werde von den Vektoren $\mathfrak{j}$ und ϑ aufgespannt. Die orthogonalen Grundvektoren ϑ, ϑ_r, ϑ rotieren im allgemeinen Fall in konstanter Entfernung c und mit zeitlich veränderlicher Winkelgeschwindigkeit

Punkt über Skalare bedeuten Differentiationen dieser Grössen nach der Zeit im raumfesten System.

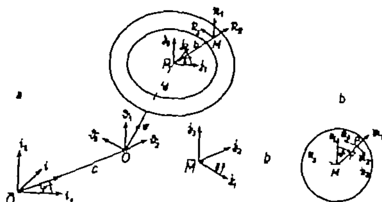


Abb. 1 Das im Inertialsystem bewegte Ringmodell mit kreisförmigem Querschnitt und ringfestem Koordinatensystem. *a*) Orthogonale Koordinatensysteme und Vektoren in Raum. *b*) Polarkoordinaten und Bezugssysteme im Ringquerschnitt

$$\vec{\omega} = \dot{\varphi}\,\mathfrak{i}_3$$

um den Vektor $\mathfrak{i}_3$ des Inertialsystems $\mathfrak{i}_1$, $\mathfrak{i}_2$, $\mathfrak{i}_3$, wobei aber der Vektor ϑ_1 stets parallel zur raumfesten Drehachse $\mathfrak{i}_3$ sein soll. Der in der Ebene $\mathfrak{i}_1$, $\mathfrak{i}_2$ liegende und von $\bar{O}$ nach O gerichtete Einheitsvektor $\mathfrak{n}$ bildet mit $\mathfrak{i}_1$ den zeitlich veränderlichen Winkel φ und bewegt sich ebenfalls mit der Geschwindigkeit $\vec{\omega}$ um $\mathfrak{i}_3$.

Auf einen im Raum bewegten Menschen übertragen, stellt O das Atlanto-occipital-Gelenk, c dessen kürzesten Abstand von der raumfesten Drehachse, $\bar{M}$ den Mittelpunkt von einem der Bogengänge und d dessen Entfernung vom Atlanto-occipital-Gelenk dar.

4. *Die Trägheitskräfte im bewegten Bogengangsmodell*

Nachdem im vorangegangenen Abschnitt die Bewegung des Bogengangsmodells festgelegt wurde, sind jetzt die zwischen den Vektoren geltenden Beziehungen anzugeben. Mit diesen kann die Lage des Ringes relativ zu den Drehachsen im Raum für speziell interessierende Falle beschrieben werden. Der am Ende in seiner allgemeinen Form mitzuteilende Teilbeschleunigungsvektor $\mathfrak{T}$ und der Partialdruck q werden sich später entsprechend der verschiedenen Einschränkungen erheblich vereinfachen.

Die Flüssigkeitsströmung in einem Ring mit kreisförmigem Querschnitt beschreibt man mathematisch einfach in einem der Geometrie dieses Torus angepassten Zylinderkoordinatensystem, dessen Zylinderachse ringförmig geschlossen ist. In den Abbildungen 1a und 1b stellen die Vektoren k_1, k_2, k_3 ein orthogonales Dreibein dar. k_3 ist gleichzeitig Tangenteneinheitsvektor der durch den Querschnittsmittelpunkt gehenden krummlinigen Zylinderachse und gibt die Richtung zunehmender Winkel ε an. In Abbildung 1b sind $\mathfrak{R}_1$, $\mathfrak{R}_2$ die Grundvektoren eines Polarkoordinatensystems im Ringquerschnitt, wobei $\mathfrak{R}_1$ den vom Kreismittelpunkt M zur Peripherie gerichteten Radiusvektor darstellt und $\mathfrak{R}_2$ die Richtung des Azimutes ϑ anzeigt. Der zu ihnen senkrechte Einheitsvektor $\mathfrak{R}_3$ ist parallel zu k_3. Sowohl der an an

derer Stelle zu berechnende Geschwindigkeitsvektor $\mathfrak{v}$ bzw. $\bar{\mathfrak{v}}$ als auch der hier interessierende Beschleunigungsvektor $\mathfrak{T}$ mögen in einem Punkt P der Ringflüssigkeit, der die Koordinaten r, ϑ, s hat, angreifen und sollen auf das letztgenannte Koordinatensystem $\mathfrak{R}_1$, $\mathfrak{R}_2$, $\mathfrak{R}_3$ bezogen werden. Während r den Abstand des Punktes P vom Querschnittsmittelpunkt M kennzeichnet, sind ϑ und s die in mathematisch positiver Richtung gewählten Winkel zwischen den Vektoren $\mathfrak{R}_1$, k_1 und k_2, j.

Mit Hilfe der Richtungscosinus können die Richtungen aller definierten Vektoren zueinander festgelegt werden. Zum Verständnis alles weiteren genügt es jedoch, nur einige als skalare Produkte in der Form

$$\delta = (\vartheta\, \mathfrak{i}_\vartheta) \qquad a_{ij} = (\mathfrak{j}\, \vartheta_j)$$

$$o = (\sigma \mathfrak{j}) \qquad c = (\mathfrak{n}\vartheta)$$

zu definieren ($i, j = 1, 2, 3$). Ist γ der Winkel zwischen der Projektion des Vektors $\mathfrak{j}$ in die ϑ, ϑ_2 Ebene und dem Vektor $\mathfrak{j}_r$, so können die Richtungscosinus a_{ij} nach Einführung der Abkürzungen

$$a_i = a_{i3}, \quad \bar{a}_i = \sqrt{1 - a_i^2}$$

als Matrix geschrieben werden, deren Elemente zum Teil den Drehwinkel φ enthalten

$$\{a_{ik}\} = \begin{pmatrix} \bar{a}_1 \cos(\varphi + \gamma_1) & \bar{a}_1 \sin(\varphi + \gamma_1) & a_1 \\ \bar{a}_2 \cos(\varphi + \gamma_2) & \bar{a}_2 \sin(\varphi + \gamma_2) & a_2 \\ \cos\varphi & \sin\varphi & 0 \end{pmatrix}$$

1 werden ferner die Abkürzungen

$$\omega_1 = \bar{a}_1 \sin\gamma_1, \qquad \omega_1 = \bar{a}_1 \cos\gamma_1$$

$$\bar{\omega}_1 = \omega_1 \cos\varphi - \omega_{11} \sin\varphi \qquad \alpha_{ij} = a_{i1}a_{j2} - a_{i2}a_{j1}$$

$$\overline{\mathrm{Re}} = \frac{a^2 b}{\nu} \dot{\varphi} \qquad \mathrm{Re} = \frac{ab}{\nu}\varphi$$

$$\mathrm{Re}' = \frac{ab}{\nu}\varphi$$

…wen let … on d nen lle letzten drei Reynolds-Zahlen darstellen, welche die Winkelgeschwindigkeiten $\dot{\varphi}$, φ und Beschleunigung $\ddot{\varphi}$ enthalten, mit denen sich der Ring im Raum bewegen kann. Definiert man η als Quotient aus Querschnittsradius a und Ringradius b ($\eta = a/b$) sowie … als die nicht grösser als 1 werdende dimensionslose Variable $r = r/a$, so ergeben sich die Komponenten T_r, T_ϑ, T des dimensionslosen Teilbeschleunigungsvektors

$$\mathfrak{T} = T_r\mathfrak{R}_1 + T_\vartheta\mathfrak{R}_2 + T\mathfrak{R}_3$$

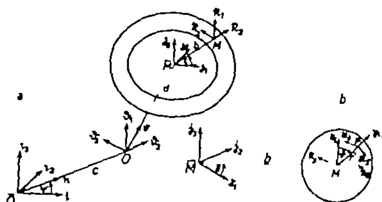


Abb. 1 Das im Inertialsystem bewegte Ringmodell mit kreisförmigem Querschnitt und ringfestem Koordinatensystem. *a*) Orthogonale Koordinatensysteme und Vektoren in Raum. *b*) Polarkoordinaten und Bezugssysteme im Ringquerschnitt.

$$\vec{\omega} = \dot{\varphi} i_3$$

um den Vektor i_3 des Inertialsystems i_1, i_2, i_3, wobei aber der Vektor ϑ_1 stets parallel zur raumfesten Drehachse i_3 sein soll. Der in der Ebene i_1, i_2 liegende und von $\bar{O}$ nach O gerichtete Einheitsvektor n bildet mit i_1 den zeitlich veränderlichen Winkel φ und bewegt sich ebenfalls mit der Geschwindigkeit $\vec{\omega}$ um i_3.

Auf einen im Raum bewegten Menschen übertragen, stellt O das Atlanto-occipital Gelenk, c dessen kürzesten Abstand von der raumfesten Drehachse, M den Mittelpunkt von einem der Bogengänge und d dessen Entfernung vom Atlanto-occipital Gelenk dar.

4. *Die Trägheitskräfte im bewegten Bogengangsmodell*

Nachdem im vorangegangenen Abschnitt die Bewegung des Bogengangsmodells festgelegt wurde, sind jetzt die zwischen den Vektoren geltenden Beziehungen anzugeben. Mit diesen kann die Lage des Ringes relativ zu den Drehachsen im Raum für speziell interessierende Fälle beschrieben werden. Der am Ende in seiner allgemeinen Form mitzuteilende Teilbeschleunigungsvektor $\mathfrak{T}$ und der Partialdruck q werden sich später entsprechend der verschiedenen Einschränkungen erheblich vereinfachen.

Die Flüssigkeitsströmung in einem Ring mit kreisförmigem Querschnitt beschreibt man mathematisch einfach in einem der Geometrie dieses Torus angepassten Zylinderkoordinatensystem, dessen Zylinderachse ringförmig geschlossen ist. In den Abbildungen 1 a und 1 b stellen die Vektoren k_1, k_2, k_3 ein orthogonales Dreibein dar. k_2 ist gleichzeitig Tangenteneinheitsvektor der durch den Querschnittsmittelpunkt gehenden krummlinigen Zylinderachse und gibt die Richtung zunehmender Winkel ε an. In Abbildung 1 b sind $\mathfrak{R}_1$, $\mathfrak{R}_2$ die Grundvektoren eines Polarkoordinatensystems im Ringquerschnitt, wobei $\mathfrak{R}_1$ den vom Kreismittelpunkt M zur Peripherie gerichteten Radiusvektor darstellt und $\mathfrak{R}_2$ die Richtung des Azimutes ϑ anzeigt. Der zu ihnen senkrechte Einheitsvektor $\mathfrak{R}_3$ ist parallel zu k_2. Sowohl der an an

$$(1+\eta r \sin\vartheta)(a \quad \cos\varepsilon + a_{s}\sin\varepsilon)$$

$$-\frac{d}{a}\overline{\mathrm{Re}}\sum_{1}^{s} a_{i}\{a \quad \eta r\cos\vartheta + (1+\eta r\sin\vartheta)(\bar{a}_{ii}\cos\varepsilon + \bar{a}_{in}\sin\varepsilon)\}$$

$$\frac{d}{b}\sum^{s} a_{i}(a \quad \mathrm{Re}^{*} + 2a_{n}\,\mathrm{Re}\,\mathrm{Re}\;)(1+\eta r\sin\vartheta)(a_{1}\cos\varepsilon + a_{s}\sin\varepsilon)$$

$$+\sum\left\{\frac{c}{a}\overline{\mathrm{Re}}(\delta_{i} \quad e \quad {}_{1} - \delta_{i} \; {}_{1}e \quad) + \frac{1}{b}\mathrm{Re}^{s}(ce - d\delta_{i}\sum_{j}^{s} a_{j}a_{n})\right\}$$

$$\{a_{in}\eta r\cos\vartheta + (1+\eta r\sin\vartheta)(a \quad \cos\varepsilon + a_{in}\sin\varepsilon)\}$$

Sämtliche Zentrifugalkräfte die an den Faktoren Re² und Re'² erkennbar sind, gehen also nur in den Druck q aber nicht in den Beschleunigungsvektor $\mathfrak{T}$ ein. Sie haben demnach keinen Einfluss auf die Geschwindigkeit der strömenden Flüssigkeit im Ring, sondern vergrössern nur den Druck in ihr. Die Strömung im Ring ist folglich unabhängig vom Abstand des Ringes von der Drehachse. Würde man statt der angenommenen Konstanz der aus Abbildung 1 ersichtlichen Entfernungen c d den ersten und zweiten Differentialquotienten dieser Grössen nach der Zeit als nicht verschwindend voraussetzen so lieferte dies nur einen Beitrag zum Druck q aber nicht zu den Komponenten T_r, T_ϑ, T_ε. Da auch die Gravitation nur in den Druck q eingehen wurde wurde sie vernachlässigt. Andernfalls müsste sie schon beim ruhenden Menschen eine Endolymphverschiebung in den Bogengängen und damit einen Nystagmus zur Folge haben.

Der Teilbeschleunigungsvektor $\mathfrak{T}$ und der Partialdruck q sind ebenfalls wie der Geschwindigkeitsvektor $\mathfrak{v}$ bzw. $\mathfrak{v}$ Funktionen der Raum Zeit Koordinaten ϑ ε τ. $\mathfrak{T}$ und q enthalten nunmehr lediglich bekannte Parameter wie die Abmessungen a und b des Ringes in Form der Reynolds-Zahlen die Dichte und Zähigkeit der Flüssigkeit, die Winkelgeschwindigkeiten $\mathfrak{r}$ und Beschleunigung $\mathfrak{f}$ des Ringes sowie die zwischen den einzelnen Vektoren bestehenden Richtungscosinus und Entfernungen. Letztere werden später dazu dienen einige im Hinblick auf die menschlichen Bogengänge besonders interessante Lagemöglichkeiten des Ringes im Raum zu verifizieren und für diese speziellen Fälle die Geschwindigkeit $\mathfrak{v}$ mit der sich die Flüssigkeit relativ zur Ringwand bewegt, zu berechnen.

SUMMARY

Basic formulae have been presented whereby the velocity of fluid flow and the pressure in a moving model of the semicircular canals can be calculated in terms of toroidal coordinates and of time. The properties and peculiarities of this model system of rings are discussed with reference to the human vestibular system. The acceleration can be calculated from the position and motion of the rings in space. Because of its inertia the fluid in the canals is affected by this acceleration and a suitable term is written into the basic formulae. Various forces which do not lead

in der folgenden Form

$$T = -\overline{\mathrm{Re}}\,(a_{11}\sin\varepsilon - a_{21}\cos\varepsilon)\cos\vartheta$$
$$-2\eta\,\mathrm{Re}\,\mathrm{Re}\;(1+\eta r\sin\vartheta)(\widetilde{\omega}_1\sin\varepsilon - \widetilde{\omega}_2\cos\varepsilon)\cos\vartheta$$
$$-2\sum_1^\infty\{(a_{11}\mathrm{Re}+a_1\mathrm{Re}\;)(w\quad_1\cos\vartheta\cos\varepsilon+v\quad_1\sin\varepsilon)-a_{21}\mathrm{Re}\,w\quad_1\sin\vartheta$$
$$+(a_{21}\mathrm{Re}+a_2\mathrm{Re}\;)(u\quad_1\cos\vartheta\sin\varepsilon-v\quad_1\cos\varepsilon)\}\eta$$

$$T_\vartheta = \overline{\mathrm{Re}}\,(a_{11}\sin\varepsilon - a_{21}\cos\varepsilon)(\eta r+\sin\vartheta)$$
$$+2\eta\,\mathrm{Re}\,\mathrm{Re}\;(1+\eta r\sin\vartheta)(\widetilde{\omega}_1\sin\varepsilon-\widetilde{\omega}_2\cos\varepsilon)\sin\vartheta$$
$$+2\sum_1^\infty\{(a_{11}\mathrm{Re}+a_1\mathrm{Re}\;)(w\quad_1\sin\vartheta\cos\varepsilon+u\quad_1\sin\varepsilon)+a_{21}\mathrm{Re}\,w\quad_1\cos\vartheta$$
$$+(a_{21}\mathrm{Re}+a_2\mathrm{Re}\;)(w\quad_1\sin\vartheta\sin\varepsilon-u\quad_1\cos\varepsilon)\}\eta$$

$$T = \overline{\mathrm{Re}}\,(a_{11}\cos\varepsilon+a_{21}\sin\varepsilon)\,\eta r\cos\vartheta$$
$$-(\widetilde{\omega}_2\eta\,\mathrm{Re}\,\mathrm{Re}\;+a_{21}\overline{\mathrm{Re}})(1+\eta r\sin\vartheta)$$
$$+2\sum_1^\infty\{[(a_{11}\mathrm{Re}+a_1\mathrm{Re}\;)\cos\varepsilon+(a_{21}\mathrm{Re}+a_2\mathrm{Re}\;)\sin\varepsilon](u\quad_1\cos\vartheta-v\quad_1\sin\vartheta)$$
$$-a_{21}\mathrm{Re}\,(u\quad_1\sin\vartheta+v\quad_1\cos\vartheta)\}\eta$$

In diesen Formeln wurden jene Summanden welche die Komponenten $u\ v\ w$ des dimensionslosen Geschwindigkeitsvektors

$$\mathfrak{v} = u\mathfrak{R}_1 + v\mathfrak{R}_2 + w\mathfrak{R}_3$$

enthalten entsprechend der nachstehenden Gleichung in Potenzreihen nach dem Parameter η entwickelt

$$(u\ v\ w\ T\ T_\vartheta\ T\;) = \sum_0^\infty(u\quad v\quad w\quad T\quad T_\vartheta\quad T\;)\ \eta \qquad (5)$$

Berücksichtigt man die zum Teil spezielleren Annahmen in den früheren Mitteilungen (Grohmann 1962 1963) so können aus der Komponente T die dort gewonnenen Ergebnisse abgeleitet werden

Während der Beschleunigungsvektor $\mathfrak{T}$ die Strömung im Bogengangsmodell bewirkt verursacht der dimensionslose Druckanteil q keine Verschiebung der Flüssigkeit

$$q = \tfrac{1}{2}(\mathrm{Re}^2+\mathrm{Re}'^2)\left\{\eta^2r^2\cos\vartheta+(1+\eta r\sin\vartheta)\left[1+\eta r\sin\vartheta+2\frac{d}{b}(o_1\cos\varepsilon+o_2\sin\varepsilon)\right]\right\}$$
$$-\tfrac{1}{2}\mathrm{Re}^2\{a_{21}\eta r\cos\vartheta+(1+\eta r\sin\vartheta)(a_{11}\cos\varepsilon+a_{21}\sin\varepsilon)\}^2$$
$$\tfrac{1}{2}\mathrm{Re}'^2\{(1+\eta r\sin\vartheta)(a_1\cos\varepsilon+a_2\sin\varepsilon)\}^2$$
$$-2\,\mathrm{Re}\,\mathrm{Re}\;\{\tfrac{1}{2}(1+\eta r\sin\vartheta)(a_{11}\cos\varepsilon+a_{21}\sin\varepsilon)+a_{21}\eta r\cos\vartheta\}$$

$$(1 + \eta r \sin \vartheta)(a_1 \cos \varepsilon + a_2 \sin \varepsilon)$$

$$- \frac{d}{a} \overline{\mathrm{Re}} \sum o_i (\bar{a} \ \eta r \cos \vartheta + (1 + \eta r \sin \vartheta)(\bar{a}_{i1} \cos \varepsilon + \bar{a}_{i2} \sin \varepsilon))$$

$$\frac{d}{b} \sum_i o_i (a_i \mathrm{Re}'^2 + 2 a_{i1} \mathrm{Re}\, \mathrm{Re})(1 + \eta r \sin \vartheta)(a \ \cos \varepsilon + a_2 \sin \varepsilon)$$

$$- \sum \left\{ \frac{c}{a} \overline{\mathrm{Re}} (\dot{\delta}_i \ e \ - \dot{\delta}_i \ e \ ) + \frac{1}{b} \mathrm{Re}^2 (ce_i - d\delta_i \sum_j^3 o_j a_{j1}) \right\}$$

$$\{ a_{i3} \eta r \cos \vartheta + (1 + \eta r \sin \vartheta)(a \ \cos \varepsilon + a_{i2} \sin \varepsilon) \}$$

Sämtliche Zentrifugalkräfte die an den Faktoren Re^2 und Re'^2 erkennbar sind gehen also nur in den Druck q aber nicht in den Beschleunigungsvektor $\mathfrak{T}$ ein. Sie haben demnach keinen Einfluss auf die Geschwindigkeit der strömenden Flüssigkeit im Ring, sondern vergrössern nur den Druck in ihr. Die Strömung im Ring ist folglich unabhängig vom Abstand des Ringes von der Drehachse. Würde man statt der angenommenen Konstanz der aus Abbildung 1 ersichtlichen Entfernungen c d den ersten und zweiten Differentialquotienten dieser Grössen nach der Zeit als nicht verschwindend voraussetzen, so lieferte dies nur einen Beitrag zum Druck q aber nicht zu den Komponenten T T_ϑ, T. Da auch die Gravitation nur in den Druck q eingeben wurde wurde sie vernachlässigt. Andernfalls müsste sie schon beim ruhenden Menschen eine Endolymphverschiebung in den Bogengängen und damit einen Nystagmus zur Folge haben.

Der Teilbeschleunigungsvektor $\mathfrak{T}$ und der Partialdruck q sind ebenfalls wie der Geschwindigkeitsvektor $\mathfrak{v}$ bzw $\bar{\mathfrak{v}}$ Funktionen der Raum Zeit Koordinaten r ϑ τ. $\mathfrak{T}$ und q enthalten nunmehr lediglich bekannte Parameter wie die Abmessungen a und b des Ringes in Form der Reynolds-Zahlen d Dichte und Zähigkeit der Flüssigkeit, die Winkelgeschwindigkeiten und Beschleunigung $\dot{\mathfrak{f}}$ des Ringes sowie die zwischen den einzelnen Vektoren bestehenden Richtungscosinus und Entfernungen. Letztere werden später dazu dienen, einige im Hinblick auf die menschlichen Bogengänge besonders interessante Lagemöglichkeiten des Ringes im Raum zu verfolgen und für diese speziellen Fälle die Geschwindigkeit $\mathfrak{v}$ mit der sich die Flüssigkeit relativ zur Ringwand bewegt, zu berechnen.

SUMMARY

Basic formulae have been presented, whereby the velocity of fluid flow and the pressure in a moving model of the semicircular canals can be calculated in terms of toroidal coordinates and of time. The properties and peculiarities of this model system of rings are discussed with reference to the human vestibular system. The acceleration can be calculated from the position and motion of the rings in space. Because of its inertia, the fluid in the canals is affected by this acceleration and a suitable term is written into the basic formulae. Various forces which do not lead

to any displacement of the fluid in the model canals have been integrated to give a single partial pressure That part of the acceleration vector which gives rise to a fluid flow is resolved into components relative to a system of spatial coordinates, in which the ring is held fixed. These components and the partial pressure are made up entirely of known parameters These include the dimensions of the model semicircular canals the density and viscosity of the fluid (expressed as Reynolds numbers), the angular velocities and angular acceleration of the ring as well as the directional cosines and distances between the vectors Because the alignment of the vectors is variable it will in future be possible to study the rings under certain interesting conditions with especial reference to the human semicircular canals. Gravity is disregarded as it does not give rise to any flow within the canals. The components of the acceleration vector are functions of space time coordinates as are also the flow velocity and the pressure which can be calculated

LITERATUR

FISCHER M H. 1928 *Die Regulationsfunktion des menschlichen Labyrinths* München Bergmann

FRENZEL, H 1936/37 Funktionen des Vestibularapparates in ihrer Bedeutung für das Fliegen. *Luftfahrtmed Abh 1* 86

— 1961 Ein einfacher Drehstuhl Modellversuch zur Frage der Corioliserregung der Bogengänge bei Kopfbewegungen während der Drehung. *Arch. Ohr Nas Kehlkopfheilk 118/111* ?

GROHMANN R., 1962 Über die tangentialen Richtungen von Trägheits- und insbesondere Coriolisströmungen in einem Modell des Bogengangsystems. *Arch. Ohr Nas K hlkopfheilk., 179* 410.

— 1963. Die Richtungen von Trägheits- und insbesondere Coriolisströmungen in den menschlichen Bogengangsystemen. *HNO 11* 112. *Berlin Gottingen Heidelberg*: Springer

— 1966 *Instationäre laminare Strömungen einer zähen inkompressiblen Flüssigkeit i einem bewegten Hohlring mit kreisformigem Querschnitt.* Mitt Max Planck Inst. f Strömungsf. u d. Aerodyn. Versuchsanst., Nr 38 Göttingen.

GUEDRY F E. und MONTAGUE, E. K., 1961 Quantitative evolution of the vestibular coriolis reaction. *Aerospace Med 32* 487

SCHINDL, K 1957 *Einwirkung des Trudelvorganges auf den menschlichen Körper Z. Flugwiss., 5* 221

SCHUBERT G 1934 Labyrinthreizung durch Zusatzbeschleunigung bei Einwirkung von Zentrifugalkraft *Pflügers Arch. Ges Physiol 233* 537

Univ Hals-Nasen-Ohrenklinik Geiststr 10,
34 Gottingen Deutschland

Eingegangen am 25 Marz 1967

Acta oto-laryngologica 64 331-333 (1967)

REHABILITATION OF LARYNGECTOMIZED SUBJECTS

M. SEEMAN

Prague Czechoslovakia

From the Phoniatric Clinic (Head Prof M Seeman) Medical Faculty Charles University Prague

Every laryngectomized person can be vocally habilitated. The esophageal voice is consciously trained. Its physiology and rehabilitation were described by the author in 1924. To facilitate and enhance the voluntary production of an esophageal ructus, transnasal insufflation of air into the esophagus is used. At the same time the tension at the upper esophageal orifice is measured because of its prognostic significance. The lesser the pressure values obtained on insufflation of air into the esophagus, the better are the results of vocal rehabilitation. If the patient is unable to acquire a loud phonation, an electrolarynx is prescribed. The best results were achieved with the model manufactured by Western Electric and Bell, which permits changing of vocal pitch. Psychic rehabilitation is described and its importance stressed. Early removal of the cannula is recommended with a view to freeing the patient of feelings of disability. After partial laryngectomy or cordectomy vocal training is introduced immediately on healing of the postoperative wound to achieve the development of a substitute vocal cord through the stimulating effect of vocal vibrations. The best functional results are obtained when using Sedláček's reconstruction of the vocal cord

The rehabilitation of laryngectomized patients is as old as laryngectomy itself. It was successfully performed for the first time by Billroth in 1873, and Gussenbauer devised a phonatory prosthesis for the patient. The surgical methods currently used at laryngectomy do not fundamentally affect the quality and efficiency of the substitute voice because the entire field of operation lies above the upper oesophageal orifice, i.e. above the level of the oesophagus, which produces the substitute voice. It is only necessary to maintain the m. cricopharyngeus and its innervation provided by the n. recurrens, which form the basis of the new pseudoglottis. Since at present about 50% of patients survive the operation for more than five years, it is only right to expect the surgeon to provide, as far as the situation permits, optimal conditions for speech rehabilitation. The first and for the only one to attempt this was A. Piccechill who, in 19?7 introduced a functional reconstructive method of laryngectomy from a broad field.

In the first attempts at vocal rehabilitation it was left to chance where

and how the substitute voice called by Landois & Strübing the pseudovoice or pharyngic voice was to develop The prerequisite for a successful rehabilitation of the lost voice is a thorough knowledge of the physiology and pathophysiology of the pseudovoice A systematic investigation into the mechanism of the pseudovoice after laryngectomy has been carried out since 1919 From this study a peculiar biological phenomenon became apparent viz. that the upper portion of the digestive tract can be trained to perform the substitute function of phonation The method of training the esophagus for phonation was introduced and the newly developed substitute voice was called for the first time, "esophageal voice" (Seeman, 1919) This term was widely accepted and has been used in the literature ever since

In the years 1922–24 the origin of the esophageal voice was investigated roentgenologically and experimentally by registration of the respiratory and phonatory movements of the esophagus in healthy and laryngectomized subjects In 1924 the results of the above studies were published in the paper Experimental and clinical studies of the development of speech without larynx with special regard to the esophageal voice" in which the physiology and origin of the esophageal voice and also the principles of development of esophageal phonation were described for the first time

The complex method of rehabilitation of the voice and speech after laryngectomy was described in 1951 The method was based on the experience gained by treating 204 laryngectomized subjects To date this method has been used at the Phoniatric Clinic in Prague for the treatment of more than 700 patients It has been employed with success in other clinics also

In the past decades there has been an alarming increase in cancer of the larynx on a worldwide scale Therefore it became a social necessity to rehabilitate laryngectomized people and thus return them if possible to work in their community Many workers are co-operating in this task The literature dealing with this subject shows a lack of uniformity in methods employed by various authors and in data concerning the mode and site of development of the substitute voice

The differences are due to the use of different methods of re-education so that the substitute voice develops under dissimilar conditions Owing to a lack of knowledge of the literature some workers are convinced that they have discovered something new Others draw general conclusions from results obtained by treating an insufficient number of cases.

To be able to compare and evaluate the data of different writers Vrtička & Svoboda (1963) examined roentgenologically and experimentally 100 laryngectomized subjects, who spoke well treated at the Phoniatric Clinic in Prague In this study which was the first of its kind the writers attempted to collect standard values on the morphology and function of the individual component structures involved in the production of the esophageal voice The statistical results corroborated Seeman's findings of

1024. The writers stress that the functional phenomena need not always correspond with the anatomical structures.

During voice rehabilitation difficulties or complications sometimes occur. In 1957 the author summed them up as follows:

(1) Large infiltrates in the anterior cervical wall developing as a result of preoperative roentgentherapy.

(2) Complications due to postoperative X-ray irradiation such as burns of anterior cervical wall.

(3) Lesion of the innervation in the m. cricopharyngicus and in the upper portion of the esophagus during the operation.

(4) Spasms in the upper portions of the esophagus.

(5) Inability of the laryngectomized person to form an esophageal ructus.

The most difficult part in the production the esophageal voice is to acquire the prephonation aspiration of air into the esophagus. In 1960 the author described a method facilitating the training of the prephonation phase by using transnasal air insufflation into the esophagus in the inspiratory position of the thorax. This procedure has been used in the past few years. While eructating the insufflated air the patient immediately starts to form vowels and words. Thus the speech therapy is facilitated and shortened as the patient acquires the proprioceptive sensations necessary during actual substitute phonation.

It was observed that, in transnasal insufflation, air sometimes enters the esophagus easily, sometimes with difficulty. This was assumed to depend on the difference in muscle tension at Killian's orifice. *Therefore* the manometric pressure required for overcoming the tonic closure of the upper esophageal orifice was measured using a Politzer bag connected to a mercury manometer. Recently Vrtička, Gundermann & Petřík (1965) have fitted the tonometer gauge directly on to the Politzer bag.

After many years of routine application of transnasal insufflation into the esophagus, the author observed that this method has an additional prognostic significance. The laryngectomized patient in whom the values *range between* 10–35 mmHg (approximately 50% of cases) soon learn to speak with an acoustically good esophageal voice. If the pressure values are between 35–80 mmHg (about 30%) re-education is *more difficult* and takes longer. The new voice is strident, choked and its vibrations are coarse. If the overcoming of the closure of Killian's orifice requires a pressure higher than 80 mmHg (about 20%) the result of rehabilitation is unsatisfactory. The patient can only master two or three words of connected speech. Voice is produced with difficulty. It is choked and interrupted by cannullary squeak. If the closure of the esophageal orifice cannot be overcome by transnasal insufflation then in most cases an esophageal voice cannot be aquired.

Another fact has emerged with increased experience. If the voice deteriorates after completed rehabilitation and becomes strained, the pressure condition have to be re-examined. In case the pressure required for over

and how the substitute voice called by Landois & Strübing the pseudovoice or pharyngic voice was to develop The prerequisite for a successful rehabilitation of the lost voice is a thorough knowledge of the physiology and pathophysiology of the pseudovoice A systematic investigation into the mechanism of the pseudovoice after laryngectomy has been carried out since 1919 From this study a peculiar biological phenomenon became apparent viz that the upper portion of the digestive tract can be trained to perform the substitute function of phonation The method of training the esophagus for phonation was introduced and the newly developed substitute voice was called for the first time "esophageal voice" (Seeman, 1919) This term was widely accepted and has been used in the literature ever since

In the years 1922–24 the origin of the esophageal voice was investigated roentgenologically and experimentally by registration of the respiratory and phonatory movements of the esophagus in healthy and laryngectomized subjects In 1924 the results of the above studies were published in the paper Experimental and clinical studies of the development of speech without larynx with special regard to the esophageal voice in which the physiology and origin of the esophageal voice and also the principles of development of esophageal phonation were described for the first time

The complex method of rehabilitation of the voice and speech after laryngectomy was described in 1951 The method was based on the experience gained by treating 204 laryngectomized subjects To date this method has been used at the Phoniatric Clinic in Prague for the treatment of more than 700 patients. It has been employed with success in other clinics also

In the past decades there has been an alarming increase in cancer of the larynx on a worldwide scale Therefore it became a social necessity to rehabilitate laryngectomized people and thus return them if possible to work in their community Many workers are co-operating in this task The literature dealing with this subject shows a lack of uniformity in methods employed by various authors and in data concerning the mode and site of development of the substitute voice

The differences are due to the use of different methods of re-education so that the substitute voice develops under dissimilar conditions Owing to a lack of knowledge of the literature some workers are convinced that they have discovered something new Others draw general conclusions from results obtained by treating an insufficient number of cases.

To be able to compare and evaluate the data of different writers Vrtička & Svoboda (1963) examined, roentgenologically and experimentally 100 laryngectomized subjects who spoke well treated at the Phoniatric Clinic in Prague In this study which was the first of its kind the writers attempted to collect standard values on the morphology and function of the individual component structures involved in the production of the esophageal voice. The statistical results corroborated Seeman s findings of

1924 The writers stress that the functional phenomena need not always correspond with the anatomical structures.

During voice rehabilitation difficulties or complications sometimes oc cur In 1957 the author summed them up as follows

(1) Large infiltrates in the anterior cervical wall d veloping as result of preoperative roentgentherapy

(2) Complication due t postoperative X-ray irradiation such as burns of nterior cervical w ll.

(3) Lesion of the in ervation in the m. cricopharyngieus and in the upper portion f the esophagus during th peration

(4) Spasms in the upper porti n of th esophagus.

( ) Inability of the laryngectomized person to f rm n esophageal ructus.

The most difficult part in the production the esophageal voice is to acquire the prephonation a piration of air into the esophagus. In 1960 the author described a method facilitating the training of the prephonation phase by using transnasal air in ufflation into the esophagus in the inpiratory position of the thorax. This procedure has been used in the past few years. While eructating the in ufflated air the patient immediately tart t form vowels and words. Thus the speech therapy is facilitated and shortened as the patient acquires the proprioceptive ensations necessary during actual substitute phonation

It wa observed that, in transnasal insufflation air sometimes enters the esophagus easily sometimes with difficulty This was assumed to depend on the difference in muscle tensi n at Killian s orifice Therefore, the manometric pressure required for overcoming the tonic closure of the upper esophageal orifice was measured using a Politzer bag connected to mercury manometer Recently Vrtička, Cundermann & Petřík (1965) ha e fitted the tonometer gauge directly on to the Politzer bag.

After m ny years of routine application of transnasal insufflation into the esophagus, the author observed that this method has an additional prognostic ignificance The laryngect mized patients in whom the values range between 10–35 mmHg (approximately 0% of cases) soon learn to spe k with n acu tically good esophageal voice If the pres ure alues are between 35 80 mmHg (about 30%) re-education i more difficult and takes longer Th new voice is trid nt, choked and its vibrations are coarse If the vercoming of the closure of Killian s orifice requires a pres u higher than 80 mmHg (about 20%) the result *of rehabilitation* are unsat sfact ry The patient can only master two or three word of connected peech. Voice i produced with difficulty it is choked and i t rrupted by cannullary squeak If the closure of the esophageal orific nn t be ercome by tran n sal in ufflation, then in most cases an esophageal voic cannot be aquired

Another f ct ha emerged with increased experience If the voice d teri r tes fter completed rehabilitation and becomes strained, the pressure condition h s e t be re-examined In case the pres ure required f r over

coming the resistance at the esophageal orifice is considerably higher than the one originally recorded a recurrence of tumor growth in the area of the upper esophageal orifice may be suspected and a roentgenographic and endoscopic control examination should be performed without delay

Summing up the conditions for an acoustically good substitute voice are as follows

(1) The pseudoglottis must be capable of voluntary contraction and thus of raising or lowering the pitch of the voice It was found that an enhanced phonation effort contracts the upper portion of the esophagus in healthy persons also (Stupka 1921) Graphic registration of phonation contractions in the esophagus of healthy and laryngectomized subjects performed by Seeman showed that they are indeed active phonation contractions

(2) The development of an air bag is essential since this serves as an air reservoir which elicits phonation in the pseudoglottis During respiration the esophagus *performs respiratory movements parallel* to the widening and narrowing of the thorax registered graphically for the first time by Dahlmann & Seeman When air is aspirated into esophagus, it initially *fills the esophagus down to its lower portions though it never reaches the* stomach as assumed by Stern (1929) and Kenessey (1957) The author together with Kohler *proved in 1924 that the air from the gastric air bubble does not participate in phonation* In laryngectomized patients whose speech is of good quality the esophagus fills with air in its upper two thirds only and the air is expressed during phonation from the esophagus by retrograde contraction of the esophageal walls (Seeman, 1960 Schlosshauer & Möckel 1954 and others) It was found that only when these retrograde contractions of the esophagus develop does there occur a jump-like improvement in the quality of esophageal speech

For re-education in esophageal voice production the laryngectomized person has to learn to aspire air into the esophagus prior to phonation. This is achieved by two simultaneous actions. By raising the hyoid as during swallowing the prelaryngic musculature influences the m crico-pharyngicus and the tonically closed upper orifice of the esophagus opens. At the same time the sudden inspiratory movement of the diaphragm increases the volume of the thoracic cavity thus causing a negative pressure in the esophagus Owing to atmospheric pressure, air is sucked into the widened esophagus Perfect training of the laryngectomized patient in the aspiration mechanism enables him later on to draw in prephonation air into the esophagus by inspiratory movement just as he used to do prior to the operation The filling of the esophagus with air by means of swallowing movements is nonphysiological and should therefore not be allowed

It is still necessary to mention the apparent contrast between the aspiration method as used at the *Phoniatric Clinic and the so-called* inhalation or injection method as described by Moolenaar-Bijl (1953) and van den

Berg These authors use in esophageal speech therapy syllables with explosive sounds mainly of the third articulation area and claim that, owing to the retraction of the tongue root, air is pressed into the esophagus and immediately expressed again In the present writer's view the pressing of air into the esophagus is not an active *movement* It seems more probable that air is sucked passively into the esophagus as a result of the negative pressure that develops both in the thorax and in the esophagus *during its deep inspiratory position*

(3) For good esophageal speech to develop it is important to achieve a continuous and easy prephonation aspiration and expression of air This is attained if the patient keeps the chest in an inspiratory position consciously and systematically during the whole act of phonation, i.e. during inspiration and expiration This makes possible an easy aspiration of air into the esophagus as well as an acceleration of the retrograde movements of the esophageal walls during articulation because the movements of the esophageal walls are undisturbed by the expiratory increase in intrathoracal pressure developing during phonation in healthy persons One of the main point in voice re-education is to interrupt the habitual phonation movements and substitute and strengthen the mechanisms which are necessary for the formation of the substitute voice

The psychic rehabilitation of laryngectomized subjects is equally important as voice rehabilitation A permanent battle has to fought against dejection and inferiority feelings. It has proved of advantage to show the patient prior to surgery and at the beginning of voice therapy some laryngectomees who are good speakers Preoperative training of esophageal phonation as recommended by some American workers, does not seem advisable for psychological reasons. The unfamiliar sound of esophageal phonation depresses the patient unnecessarily causing neurosis It should also be borne in mind that postoperatively the patient is unable because of contriction of the vocal cord to produce the excess pressure in his thoracical cavity required for esophageal phonation It was found that certain laryngectomees who were able to eructate prior to surgery were unable to do so afterwards. If vocal reeducation is difficult, the laryngectomee need constant encouragement on the part of the physician. *The cooperation of the patient family is also of great importance* They should appear pleased with his progress. In younger individual the prospect of a return to work is a potent stimulus Němec & Vrtička (1962) have demonstrated statistically significant correlation between the following quality of voice and sensory engagement, quality of voice and age quality of speech and intelligence

Early removal of the cannula contributes to psychic rehabilitation in spite of the difficulty caused by plastic covering of the wide tracheostoma. The patient gets rid of all the discomfort associated with carrying and cleaning of the cannula and also *more quickly of his feeling of disablement*

On rare occasions, when the patient does not learn to phonate loudly

an electrolarynx is used for the production of a loud phonation. It is attached to the anterior cervical wall The best results were achieved with the new model of the firm Western Electric and Bell The laryngectomee can change the pitch of the voice by pressing the lever of the instrument with his finger In this way unpleasant monotony of voice is avoided. This effect cannot be achieved with the appliance "pipa" carried like a pipe in the mouth (manufactured by Ticchioni in Milano) The results of experiments with the pipa were not satisfactory Pichler devised a new principle for an artificial larynx. He used a miniature sound reproducer fixed to an upper denture acting as a sound emitter controlled by an intricate wireless apparatus. The idea is very good but the present model is not suitable for practical purposes

After partial laryngectomy or chordectomy careful vocal training is started immediately on healing of the laryngofissure The phonation vibrations of the healthy vocal cord are a stimulus for the development of a good shape of the substitute cord Thus it is often possible to produce a voice that has a good sound and is resistant. A fully normal voice is achieved by the operative procedure of Sedláček who replaces the removed cord by a catgut thread connecting the arytenoid cartilage to the anterior commisure This is covered by mucous membrane This procedure may be also used at partial resection of vocal cords The method of Sedláček, though very simple has given surprisingly good results

ZUSAMMENFASSUNG

Es ist möglich jeden Laryngektomierten stimmlich zu rehabilitieren Wir üben systematisch die Ösophagusstimme ein deren Pathophysiologie und Rehabilitation der Autor bereits 1924 beschrieben hat Zur Erleichterung und Beschleunigung der willkürlichen Bildung des Ösophagusructus wird jetzt methodisch eine transnasale Insufflation von Luft in die Speiserohre angewendet Dabei messen wir gleichzeitig mittels eines Manometers die Kraft des Verschlusses des oberen Ösophagusmundes, was auch eine prognostische Bedeutung hat Je geringer die dabei festgestellten Druckwerte sind desto besser ist der Erfolg der entgültigen stimmlichen Rehabilitation Wenn der Patient nicht laut zu phonieren lernen kann verschreiben wir einen Elektrolarynx Am besten hat sich bei uns das neue Modell der Firma Western Electric and Bell bewährt das eine Änderung der Tonhöhe ermöglicht Es wird die Bedeutung und die Art der physischen Rehabilitation beschrieben Empfohlen wird ein baldiges Dekanulement damit der Patient die Gefuhle seiner Invalidität verliert Nach einer partiellen Laryngektomie oder Chordektomie beginnen wir mit den Stimmubungen sofort nach der Wundheilung, damit sich durch die stimulierende Wirkung der Stimmvibrationen eine gute Ersatzstimmlippe ausbildet Die besten funktionellen Resultate wurden durch die Rekonstruktion der extirpierten Stimmlippe nach Sedláček erreicht

REFERENCES

DOLMEN, P 1962 Sprachbildung bei Laryngektomierten. *Mschr Ohrheilk.*, 96 280

JARVSVEY L., 1957 *Fül-Orr'Gége-Gyogy-aszat* 9

MOOLENAAR-BIJL, A., 1953 Connection between consonant articulation and the intake of air in oesophageal speech. *Folia Phoniat (Basel)* 5, 212.

NĚMEC, J and VRTIČKA, K., 1962: Główne czynniki psychologiczne wpływające na rehabilitację głosu i mowy u pacjentów po przebytej laryngektomii *Logopedia* (Lublin) 3, 103

PICHLER, H., 1961 Über ein neuartiges atomatisch gesteuertes elektronisches Sprechgerät für Laryngektomierte *Acta Otolaryng* (Stockh.) 53 374

PÉRCHCURRELL, A 1950 Reconstitution fonctionnelle de la pseudo-glotte oesophagien dans la laryngectomie. *Acta Oto-Rhinolaryng Belg* 6 550

SCHLOSSHAUER, B., und MÖCKEL, G 1954 Röntgenkinematographische Darstellung der Pseudosprache nach Laryngektomie *Arch Ohr Nas. K hlk pfheilk* 165 581

SEEMAN, M 1922 Speech and voice without larynx. *Cas lék čes.*, 41 369

1924 An experimental and clinical study of the development of speech without larynx. *Cas. lék. čes.*, 43 984

1926 Phoniatrische Bemerkungen zur Laryngektomie *Arch. Klin Chir* 140 285.

1927 Registrierung ösophagealer Phonationsbewegungen. V (Hamburg) 9 23.

1958 Z Pathologie der Ösophagusstimme *Fol Ph niat* (Basel) 10 44

1960 Die Bedeutung der Kraft des Verschlusses des Ösophagusmundes für die Rehabilitation der Stimme nach Laryngektomie. *Kongressbericht der Gemeinschaftstagung für allgemeine und angewandte Phonetik* Hamburg

— 1961 Nouveaux résultats des recherches expérimentales dans la rééducation de la voix oesophagienne *J Franç Oto-rhino-laryng* 10 47

STERN H 1929 Sprech- u. Stimmbildung bei Laryngektomierten. *Hdb Hals. Nasen. Ohrenheilk* 5. Berlin.

STURMANN, W 1921 Über ein Phonationsphänomen an den Speisewegen. *Mschr Ohrenheilk* 55, 1494

VRTIČKA, K G ... und PERKLE, M., 1965 Ein faches Messgerät zur Feststellung der Spannung des oberen Oesophagusmundes bei Laryngektomierten n. *HNO-Wegweiser* Berlin 13 175

VRTIČKA, K., and SVOBODA, M 1961 A clinical and X-ray study of 100 laryngectomized speakers *Folia Phoniat* (Basel) 13, 174

1963 Time changes in the X-ray picture of the hypopharynx, pseudoglottis and oesophagus in the course of vocal rehabilitation in 70 laryngectomized speakers *Folia Phoniat* (Basel) 15, 1

Vdbr B Engelse 68, Prague
Czechoslovakia

Received January 9 1967

an electrolarynx is used for the production of a loud phonation. It is attached to the anterior cervical wall The best results were achieved with the new model of the firm Western Electric and Bell The laryngectomee can change the pitch of the voice by pressing the lever of the instrument with his finger In this way unpleasant monotony of voice is avoided. This effect cannot be achieved with the appliance "pipa" carried like a pipe in the mouth (manufactured by Ticchioni in Milano) The results of experiments with the pipa were not satisfactory Pichler devised a new principle for an artificial larynx He used a miniature sound reproducer fixed to an upper denture acting as a sound emitter controlled by an intricate wireless apparatus The idea is very good but the present model is not suitable for practical purposes.

After partial laryngectomy or chordectomy careful vocal training is started immediately on healing of the laryngofissure The phonation vibrations of the healthy vocal cord are a stimulus for the development of a good shape of the substitute cord Thus it is often possible to produce a voice that has a good sound and is resistant A fully normal voice is achieved by the operative procedure of Sedláček who replaces the removed cord by a catgut thread connecting the arytenoid cartilage to the anterior commisure This is covered by mucous membrane This procedure may be also used at partial resection of vocal cords. The method of Sedláček, though very simple, has given surprisingly good results

ZUSAMMENFASSUNG

Es ist möglich jeden Laryngektomierten stimmlich zu rehabilitieren Wir üben systematisch die Ösophagusstimme ein deren Pathophysiologie und Rehabilitation der Autor bereits 1924 beschrieben hat Zur Erleichterung und Beschleunigung der willkürlichen Bildung des Ösophagusructus wird jetzt methodisch eine transnasale Insufflation von Luft in die Speiseröhre angewendet Dabei messen wir gleichzeitig mittels eines Manometers die Kraft des Verschlusses des oberen Ösophagusmundes, was auch eine prognostische Bedeutung hat Je geringer die dabei festgestellten Druckwerte sind desto besser ist der Erfolg der entgültigen stimmlichen Rehabilitation Wenn der Patient nicht laut zu phonieren lernen kann verschreiben wir einen Elektrolarynx Am besten hat sich bei uns das neue Modell der Firma Western Electric and Bell bewährt, das eine Änderung der Tonhöhe ermöglicht. Es wird die Bedeutung und die Art der physischen Rehabilitation beschrieben Empfohlen wird ein baldiges Dekanulement damit der Patient die Gefühle seiner Invalidität verliert Nach einer partiellen Laryngektomie oder Chordektomie beginnen wir mit den Stimmubungen sofort nach der Wundheilung damit sich durch die stimulierende Wirkung der Stimmvibrationen eine gute Ersatzstimmlippe ausbildet Die besten funktionellen Resultate wurden durch die Rekonstruktion der extirpierten Stimmlippe nach Sedláček erreicht

apparatus had only to follow up their work. The lacrimal fossa is situated in the lower part of the medial orbital margin at the level of the medial part of the nasal cavity. From its lower portion the nasolacrimal duct departs, the mouth of which is situated between the first and second thirds, roughly speaking, of the inferior concha. The membraneous parts of the nasolacrimal duct are thus surrounded by bony structures. The lacrimal sac into which the canaliculi open out, is situated in the lacrimal fossa and is completely surrounded by periosteum. The sac measures about 12 mm and is directly continued by the nasolacrimal duct, the intraosseous part of which measures about 12.5 mm while its lower part, situated in the mucosa of the lateral wall of the nasal cavity measures about 3.5 mm. These measures show however a wide variation. The mouth of the nasolacrimal duct too varies widely with regard to site and shape. In most cases it is situated laterally to the inferior concha, usually some 30–40 mm behind the nostril. The lacrimal sac and the nasolacrimal duct are coated with two-laminar columnar epithelium. This is adjacent to the substantia propria and consists of a layer of adenoid tissue resting on a fibrous layer containing an abundance of elastic fibres. At the mouth of the nasolacrimal duct there is a fold of mucous membrane, Hasner's valve, which is regarded as being of importance to the physiology of the lacrimal apparatus.

In the lacrimal apparatus the passage of the tear secretion to the nose is promoted by an active physiologic process, acting like a pump mechanism. Jones & Boyden have given an excellent description of the various muscles and ligaments which in connection with the eyelids, function as active forces in the operation of the lacrimal passages. Closure of the eyelid causes shortening of the canaliculi and a simultaneous movement of the lateral wall of the lacrimal sac which leads to a decreased pressure in the latter with a resulting inflow of secretion. When the lid is opened the canaliculi are extended, the lacrimal sac is compressed and the tears are pressed down into the nasolacrimal duct. Epiphora may be due to blockage of the lacrimal passages or to malposition of the lower lid in particular or to a disturbance in the physiological function of the lacrimal passages.

PROBLEMS

The present study was undertaken in order to throw light on the following points:

1. The occurrence and causes of epiphora after maxillary resection and irradiation for carcinoma. Particular attention was paid to the part possibly played by the operation and irradiation.

2. The possibility of performing the surgery so as to avoid future complications from the lacrimal apparatus.

MATERIAL AND METHODS

Questionnaires relating to postoperative troubles in the form of epiphora were distributed to 43 patients on whom maxillary resection had been performed at the Department of Otolaryngology, Helsinki University Central Hospital during the years 19[illegible]6–1963. A total of 40 patients replied

Acta oto-laryngologica 64 242 250 (1967)

EPIPHORA AFTER MAXILLARY RESECTION

U Engzell[1] and G Johnsson
Helsinki Finland

From the Department of Otolaryngology (Head Prof U Siirala) Helsinki University Central Hospital Helsinki

A follow-up study was performed on patients treated with maxillary resection and irradiation for maxillary cancer particular attention being paid to the function of the lacrimal passages and the causes of epiphora. A questionnaire was replied to by 19 patients and 10 of these complained of epiphora 9 patients had a clinical follow-up examination One of them exhibited no epiphora because of the creation of a wide communication between the lacrimal sac and the operative cavity during the radical operation The probable causes of epiphora in 8 patients followed-up were (1) A lesion of the nasolacrimal duct sustained at the operation (3 cases) (2) A functional disturbance of the canaliculi in spite of patent lacrimal passages (2 cases) (3) Stenosis of the lacrimal punctum (1 case) (4) Ectropium (1 case) (5) Stenosis of the lacrimal punctum and ectropium (1 case) In 3 cases the postoperative irradiation was probably responsible for the development of epiphora. In selected cases endonasal dacryocystorhinostomy ought to be performed primarily in connection with the maxillary resection If functioning canaliculi are preserved epiphora may be secondarily eliminated by canaliculorhinostomy

After partial or total resection of the maxilla, involving the extirpation of a proportion of the medial wall of the maxillary cavity some patients are postoperatively troubled with epiphora No investigations seem to have been performed, however concerning this complication and its immediate causes At the Department of Otolaryngology Helsinki University Central Hospital we therefore decided to undertake a follow up study of patients operated on for malignant processes involving mainly the maxillary cavity but also the ethmoidal region in order to elucidate the occurrence and causes of postoperative epiphora

Anatomy

As early as 1574 Giovanni Battista Carcano Leone described the nasolacrimal duct and when the Danish anatomist Niels Steensen in 1662 had clarified the function of the lacrimal glands, later investigators of the anatomy of the lacrimal

[1] Present address: ENT Department, Karolinska Sjukhuset, Stockholm 60, Sweden.

Table 2. Data regarding the patients examined

Pat. no.	Sex	Age	Diagnosis	Incision	Status of maxilla	Year of op.	Dosage of postop. irradiation	Ectropion	Epiphora	Passage at dacryocystography
1	m	63	Ca sinus maxillar. d.	Skin (Fergusson)	Extirp.	1956	X-ray 5181			N (sac filled)
2	f	71	Ca sinus maxillar. sin.	Mucous (oral)	Extirp.	1957	X-ray 0003			N
3	m	59	Ca sinus maxillar. d.	Skin (Fergusson)	Extirp.	1959	Cobalt, 3000 r X-ray 4221		+ + +	N (sac not filled)
4	m	70	Ca sinus maxillar. dx.	Skin (Fergusson)	Extirp.	1961	X-ray 5288 r 3150		+ +	Yes (Stenosis of lower lacrimal punctum)
5	m	16	Ca sinus maxillar. et ethmoidal. d.	Skin (Fergusson)	Extirp.	1962	Type and dosage of irradiation known			Yes (narrow passage)
6	m	73	Ca sinus maxillar. et palat. dur. amb.	Skin (Fergusson)	Not extirp.	1962	Gammatron, 6015 X-ray 318 r		+	Yes
7	m	52	Ca sinus maxillar. et ethmoidal. sin.	Skin (Fergusson)	Extirp.	1962	Gammatron, 0528 r X-ray 180		–	Yes (dacryocystorhinostomy made at prim. operation)
8	m	67	Ca sinus maxillar. d.	Skin (Fergusson)	Not extirp.	1963	X-ray 1801 r	+	+ + +	Yes (Stenosis of lower lacrimal punct.)
9	m	70	Ca sinus maxillar. d.	Skin (Fergusson)	Extirp.	1963	Cobalt 5200		+ + +	Yes (No spontaneous passage of fluorescein. Slow function of the eye lid.)

TABLE 1 *Occurrence of epiphora after maxillary resection and irradiation treatment for carcinoma in 19 cases*

	Epiphora	No epiphora	Total
Patients examined	8	1	9
Patients only questioned	2	8	10
Total	10	9	19

Dacryocystorhinostomy was performed in connection with maxillary resection

Of these 9 were available for a follow up study In all of the 19 cases partial maxillary resection had been performed The eye and the orbital floor of the maxilla had been left as intact as possible except in one case in which the entire orbital floor had been extirpated All patients received postoperative irradiation Both in the questionnaires and at the follow-up examinations particular attention was paid to the history of epiphora. None of the 19 patients had had epiphora prior to operation. With regard to the condition subsequent to the treatment the patients were questioned as to whether they had been troubled with unilateral epiphora on the operated side and whether epiphora—if it had occurred—had developed after the operation or after the irradiation treatment. All the 9 patients who attended at follow up examinations were investigated at the Department of Otolaryngology Helsinki University Central Hospital In all cases the operative area was inspected with special attention paid to the position and function of the eyelids, and in 8 cases dacryocystography was performed In one case fluorescein was used as a contrast medium in investigating the lacrimal passages

RESULTS

Of the present 19 patients 10 had experienced epiphora postoperatively (Table 1) The data regarding all patients are compiled in Tables 2 and 3

Patients without Epiphora

Only one of the 9 patients who were subjectively symptom free attended at the follow up study In 4 patients in this group the medial wall of the maxillary cavity had been left intact 5 patients were operated on via incisions in the oral cavity while Fergusson s skin incision was made in 3 cases 4 patients were asymptomatic although large portions of the anterior and medial walls of the maxillary cavity including the inferior concha had been extirpated Of these 2 had been operated on via an incision in the oral cavity and 2 via external incisions of Fergusson s type

The only patient without epiphora, who attended had been operated on

T a l 2 *Dat regarding th patient xamined*

Pat. no.	Sex	Age	Diagnosed	Incision	Stat of med ll	Yea of op.	Dosage of postop irradiation	Ectropi m	Epiphora	Passage t dacryocy to-graphy
1	m	65	Ca sinu maxillar dx	Skin (Fergusson)	Extirp	1956	X-ray 5181	–		No (sac filled)
2	f	71	Ca sinus maxillar si	Mucou (oral)	Extirp	1957	X-ray 6063		+ +	N
3	m	59	Ca sinus maxillar dx	Ski (Fergusson)	Extirp	1959	Cobalt 3000 r X-ray 4221		+	N (sac not filled)
4	m	70	Ca sinu maxillar d	Skin (Fergusson)	Extirp.	1961	X-ray 5288 r 3150		+	Yes (Stenosis of lower lacrim punct m)
5	m	16	Ca sinus maxillar t ethmoid d	Skin (Fergusson)	Extirp.	1962	Type nd dosage f irradiation know		+	Yes (narrow passage)
6	m	77	Ca sinus maxillar t palat d mb	Ski (Fergusson)	Not extirp.	1962	Gammatron 6015 r X-ray 318		+	Yes
7	m	52	Ca sinus maxillar et ethmoid si	Skin (Fergusson)	Extirp.	1962	Gammatron, 4526 r X-ray 189			Yes (dacryocystorhino-stomy made t prim. operation)
8	m	67	Ca si us maxillar d	Skin (Fergusson)	Not extirp	1963	X-ray 4801	+	+ +	Yes (Stenosis f lo la-crim punct m)
9	m	70	Ca sinus maxillar sin	Skin (Fergusson)	E tirp.	1963	Cobalt, 5200		+ + +	Yes (N pont neous pas-sage f fluorescein. Slow function f the ey lid)

in 1962. Partial maxillary resection and ethmoidectomy were made and the incision was of Fergusson's type. The medial and anterior walls and one-third of the plate of the palatine bone were extirpated. At the operation a communication was created between the lacrimal sac and the operative cavity. Postoperatively and during the irradiation treatment the patient experienced epiphora, but subsequently this symptom disappeared. Six months after the operation there was no epiphora. Dacryocystography then revealed a patent and wide passage between the lacrimal sac and the operative cavity.

Patients with Epiphora

In one of the two cases with epiphora that were not followed up (no. 12, Table 3) total resection had been performed. The orbital floor was extirpated without being substituted. According to the operative record, the position of the eye was markedly changed. In the other case (no. 13) partial resection involving extirpation of the medial wall of the maxillary sinus was performed via an external skin incision. In 8 cases (Nos. 1–6 and nos. 8 and 9) copious epiphora was present. In all these cases except one (no. 2) the operation was performed via Fergusson's skin incision, and the medial maxillary wall was extirpated except in cases 6 and 8.

The Causes of Epiphora (Table 4)

In spite of the small number of patients, the present study yields some information concerning the lesions and dysfunction of the lacrimal system occurring after radical surgery involving the medial wall of the maxillary cavity and after irradiation of the area adjacent to the eye. No conclusions regarding the frequency of these complications can be drawn. Furthermore, it may be difficult to decide whether a block is attributable to the operation or the irradiation, and it is also possible that postoperative cicatricial shrinkage, and not the irradiation, is responsible. Nonetheless, some hints can be derived from the time of onset of epiphora, whether prior to or after irradiation, which was always given postoperatively.

In case 1, in which epiphora developed immediately after operation, the lacrimal sac was filled at dacryocystography, but from the lacrimal sac to the operative cavity no contrast medium seemed to pass. In this case a block was undoubtedly present below the lacrimal sac.

In case 2 no epiphora occurred for the first three months after operation. This complication developed immediately after the completion of the irradiation treatment, which was commenced one month after the operation.

In case 3, in which postoperative epiphora soon developed, a lesion had obviously been caused by the surgery. Dacryocystography revealed intact canaliculi but there was no filling of the lacrimal sac, which presumably had been damaged.

TABLE 3 *Data regarding the patients not examined*

Pat. no	Sex	Age	Diagnosis	Incision	State of med. wall	Year of op	Epiphora
10	m	51	Ca sinus maxillar sin	Mucous (oral)	Extirp.	193	−
11	m	68	Ca sinus maxillar sin	Skin (Ferguson)	Extirp.	1959	−
12	f	60	Ca sin maxillar et ethmoid sin	Skin (Ferguson)	Extirp	1960	+
13	m	37	Ca sinus maxillar et ethmoid sin	Skin (Ferguson)	Extirp.	1961	+
14	m	54	Ret. sarc maxillar et ethmoid dx	Skin (Ferguson)	Not extirp	1961	−
15	f	73	Ca sinus maxillar l	Skin (Ferguson)	Extirp	1961	−
16	m	63	Ret. sarc. maxillar et ethmoid si	Mucous (oral)	Not extirp.	1961	−
17	f	19	T m malign ethmoid si	Mucous (oral)	Not extirp.	1962	−
18	m	69	Ca sinus maxilla et ethmoid si	Mucous (oral)	Extirp.	1962	−
1	m	61	Ca sinus maxilla sin	Skin (Ferguson)	Not extirp.	1962	

Patients 5 and 9 exhibited patent lacrimal passages both clinically and on dacryocystography although the nasolacrimal duct was not visible in case and only uncertainly visualized in case 9. In both cases the operation was performed via a skin incision running parallel with and near the margin of the lower eyelid. In case 5 epiphora set in after the irradiation treatment, in case 9 after the operation only to increase during the irradiation treatment. In this case a definitely diminished motility of the both lids was observed and fluorescein did not pass through the roentgenologically patent canaliculi and nasolacrimal duct. Either the irradiation treatment or the incision in the region of the orbicular muscle or both together must have impaired the function of the eyelids.

In cases f and 8 ectropium was observed on the operated lid and it may be assumed that this lesion was due to similar causes as was the lesion in case 9. The irradiation treatment alone may have been respon

TABLE 4 *Causes of epiphora after maxillary resection and irradiation treatment for carcinoma in 8 cases*

	No. of cases
Complete postoperative blockage of the lacrimal passages	3
Functional disturbance in spite of patent lacrimal passages	2
Blockage of lower lacrimal punctum	1
Ectropium	1
Ectropium and blockage of lower lacrimal punctum	1

sible, since both these patients exhibited dyschromia and shrinkage of the skin under the eye

Two patients (nos 4 and 8) showed blockage of the lower punctum. In these cases epiphora developed towards the end of the irradiation treatment As mentioned above the block present in case 8 was associated with ectropium

Patient no 12 was not followed up Since in this case the entire orbital floor had been extirpated and not substituted, it may be assumed that a marked change in the position of the eye with consequent effects on the lacrimal passages and possibly ectropium were the causes of the epiphora.

This paper only deals with lesions of the efferent lacrimal passages. The effect of the irradiation treatment on the tear secretion and other structures of the eye are not taken into account

DISCUSSION

In connection with radical maxillary operations the efferent lacrimal passages are often injured Apart from any direct anatomical lesions caused ectropium may ensue with resulting impairment of the lacrimal pumping system The irradiation treatment may cause stenosis of the lower lacrimal punctum in particular (Jones & Boyden, 1960) through which normally about 80 per cent of the lacrimal secretion passes. After the treatment has been completed some patients have functioning lacrimal passages however while others exhibit epiphora

Owing to the severity of the primary disease the patients as a rule pay little attention to this postoperative complication It is not much discussed in the literature either and the operation is mostly performed without any precaution taken to secure proper functioning of the efferent lacrimal passages When their lower part is admittedly injured prophylactic dacryocystorhinostomy may in selected cases be made primarily in connection with the radical operation In the only one of the present cases in which this had been done the patient had no epiphora at the last examination which was performed six months after the operation

If the lacrimal sac is severely damaged while th canaliculi remain intact, canaliculorhinostomy may be secondarily performed by methods lately described by Björk (1963) Griffith (1963) Cröndahl (1963) and others

During the irradiation treatment it is important to protect the eye very carefully and in order to av id the development of canalicular stenosis and ectr pium incisions should not be made near the interior angle of the eye or in the lid Larsson & Mårtensson (1954) emphasized that the use of ext rnal skin incisions was necessary in only a few of their 114 cases.

E en though many patients who are postoperatively troubled with epiphora, get on tolerably well, there seems to be reason for paying greater attention to the pos ibility of preserving functional lacrimal passages, now that the result of treatment of maxillary carcinoma are steadily improving.

ZUSAMMENFASSUNG

Eine kli ische Nach tersuchung ist be 1 tienten die sich M xilla Resektion und Strahlentherapie für Ma ill r-Cancer unterzogen a geführt worden wobei besondere Aufmerksamkeit auf di Funktion der Trä engänge und die Ursachen des T änenergusses gerichtet wurde Ein Frageformula wurd von 19 Patienten beantwort t 10 on ihnen klagten über Trä nerguss. 9 Patienten kamen zu ner klinischen Nachuntersu h ng iner von ihnen zeigte kein Epiphora dank der Bildung iner weiten Verbindung zw schen dem Tränensack und der Operationshöhle wäh end der radikalen Operation Die wahrscheinlichen Ursachen des T änenergu ses bei 8 klinisch nachuntersuchten Patienten w ren 1) Verlet u g des Trä enka als a f Grund der Operation (3 Fälle) 2) Funktionelle Störung der Kanälchen trotz offener T änengänge (2 Fäll) 3) Stenosierung des T nenpunctums (1 F ll) 4) Ectropion (1 F ll) 5) Stenosierung des Trä n punctum und Ectropion (1 F ll) Bei 3 Fällen war die postoperative Strahlenbehandlung für d Entw cklung des Tränen rgusses wahrscheinl h verantwortlich Endonasale D kryo torh ostomie sollt In ausgewählten Fällen im Anschlus die Ki fernoperation prim r ausgeführt werden W nn funktionierende Kanalchen bew hrt ind kann Epipl ora mit Canaliculorhi ostomie sekund r elimi iert werden

REFERENCES

Busse H 1963 Endonasale Dacry cystorhinost mie und Ka lik lorhinost mie *Arch Augenh* (Ill 97 213

Du E wen, S 1961 *Sy t m f Ophthalmology* L 2. Kimpton London.

Griffith T P 1963 Polyethylene t bes i canalicul rgery *Brit J Ophthal* 47 393

Gröndahl L. J 1963 Dacryost nosis. *Act Ophthal* (Kbh) 41 235.

Jones L T and Boyer C L, 1960 The lacrimal pparat 1 *Otolaryngology* (Coates, Schenck, Miller ol. 3, Ch 29 W F Prior Company H gerstown.

K Anrep, M 1937 On dacryostomies. *A t Ophtal.* (Kbh) 15 491

Larsson, L-G nd Mårtensson G 1954 Carcinomas of the paranasal si ses nd the

nasal cavities A clinical study of 397 cases treated at Radiumhemmet and the Ear Nose and Throat Department f Karolin ka Sjukhuset 1940–1950 Acta Radiol (Stockh) 52 140
MARTIN, H 1057: *Su gery of Head and Neck Tumours.* A Hoeber-Harper Book, New York
N HERI E., and SETÄLÄ, H 1962 Malignant tumours of paranasal inuses. A ta Oto-laryng (Stockh) 55 561
VEIRS, E. R 1955 *Lacrimal System* Grune & Stratton New York.

U Eng ell ENT Department
Karolinska Sjukhuset Stockholm 60
Sweden
G Johnss n ENT Department
Uni ersity of Hel inki
Finland

Recei ed January 2 1967

Acta oto-laryngologica 64, 231 244 (1967)

TRACHEOPATHIA OSTEOPLASTICA

EINO VAHERI and EERO VAHERI
Helsinki Finland

From the Otolaryngological Hospital (Head Prof U Siirol) University f Helsinki

Two hist logically exami ed cases of tracheopathia osteoplastica are reported A metaplastic change of th respiratory epithelium into squamou epithelium was found. The el sti cartilage in the nodules probably also arises through metaplasia from the elastic connective tissue The cartilage later undergoes calcification nd ossification

Tracheopathia osteoplastica is a relatively rare disease in which small bony hard nodules occur on the walls of the trachea and often also of the main bronchi Histological examination shows subepithelial cartilage and bone islands entirely separate from the hyaline cartilage of the trachea. Wilks wa the first to describe the disease as early as 1857 Most of the cases hithert described were discovered incidentally at autopsy since the clinical ymptoms are slight and may be absent The increasing use of bronchoscopy has led to the disease being diagnosed relatively often during lif (Jackson & Jackson, 1932 Moersch, Broders & Havens, 1937 Larsen, 1938 Clerf 1944 Schneider 1949 Carr & Olsen 1954 Padovan, 1954 Heyden 19 4 Jepsen & Sörensen, 1960 Matzker & Claus, 1963 Elmlnd, 1964)

S me cases show slight symptoms, such as a sensation of dryness in the throat hoskines f the voice cough and mild dyspnea. Most of the patient enter hospital and come under bronchoscopic examination because of bloody expectorations.

The majority are over 50 years of age No cases in children have been diagnosed Morbidity ppears to be about the same in both sexes.

The bronchoscopic findings are highly characteristic There are numer ou ses ile nodules co ered by apparently healthy mucous membrane and they occur on all tracheal wall except th membranou posterior wall. At th l te tage the lumen may be narrowed even to one third of its normal re (Moersch, Broder & H vens, 193) nd obstructive pneumonitl may dev lop a a res lt of the narrowing (Carr & Olsen, 1954) Besides broncho-scopy tomogram of th trachea and bron hi can be of value in di gnosis nd in determining the extent f the disease

CASE REPORTS

Ca 1

The patient wa farmer wif aged 50 Sl ght hemopt si had occurred twice i the course of) d th qu l t of th inging ice had deteriorated Roentgen amin tion mad seven month previously t tuberculosis sanato-

nasal cavities. A clinical study of 397 cases treated at Radiumhemmet and the Ear, Nose and Throat Department of Karolinska Sjukhuset 1940–1950 *Acta Radiol.* (Stockh.) 42 149

MARTIN, H 1957: *Surgery of Head and Neck Tumours* A Hoeber Harper Book, New York

VAHERI, E., and SETÄLÄ, H 1962: Malignant tumours of para nasal sinuses. *Acta Oto-laryng* (Stockh) 54 561

VEIRS, E. R 1955: *Lacrimal System* Grune & Stratton, New York.

U Engzell ENT Department
Karolinska Sjukhuset Stockholm 60
Sweden
G Johnsson ENT Department
University of Helsinki
Finland

Received January 27 1967



Fig. 2 Photomicrograph of bony nodule There are spicules f bone and f t tissue resembling fat marrow

Case 2

The patient wa married woman aged 63. For o er 10 ears he had been suffering from ch oni trophi hinitis, a dry cough a d crust formation in the nose but n foul odour Th crust disappeared during course of vitamin A. She had slight pistaxi for one month si day ago, in connection with an acute febrile infect on, fairly large moont of blood and clots appeared in th mouth. This discharge lasted for two days and the sputum has since been blood m xed.

Examin tion showed that the blood count was normal and sedimentation rate mm The nasal ca ities were large with some crust in the upper parts. The phary was ordinary On coughing some bloody sputum was brought up Roentgen examination Pulmonary fibrosis, no shadows referable to foci, hila shadows somewhat enlarged.

Bronchoscop examin tion, December 1963 (Vaheri, Ein & Eero) The laryn as pparently normal, th tracheal nd bronchial mucosa slightly reddened. I the lower part f the trachea, on the right wall, there was n rea consisting of sessile od les. A few nodules were lso seen laterally in the right main bron hus and in the posterior and inferior wall of the upper lobe bron h, also



FIG. 1 Case 1 Endoscopic ppearance of trachea showing numerous bony hard nod les.

rium showed slight shadows at the apex of the right lung (cured tuberculosis) At the sanatorium bronchoscopy revealed a "nodular tumour" on the right wall of the trachea and biopsy examination a mild chronic inflammation On tomographic examination of the trachea six months ago a flat protuberance was found on the right wall The patient was referred to the Otolaryngological Hospital in Helsinki

On examination the blood count was normal and sedimentation rate 5 mm. The nose and pharynx showed no findings of special importance

Bronchoscopic examination May 1960 (Vaheri Eino) The larynx trachea and bronchial mucosa were of ordinary appearance A nodular area covered by apparently normal mucous membrane and causing narrowing of the lumen was seen in the right wall about 4 cm below the rima glottidis and extended further downward The nodules varied from the size of a grain of rice to that of a large pea The left and anterior wall were also nodular but not the posterior wall A few prominences occurred on the lateral wall of the left main bronchus and the medial wall of the right main and stem bronchus. Mucus was of slight amount Two colour exposures were made (Fig 1) A mucus specimen was taken for culture of tubercle bacilli In spite of the difficulty of obtaining biopsy material from the bony hard nodules, spe imens were successfully removed from two

Histological exmination On the surface there is respiratory epithelium in places, metaplastic epithelium in places Nonspecific inflammatory infiltration (lymphocytes and plasma cells) appear subepithelially Deeper down elastic cartilage is seen and in close proximity islands of lamellated bone and marrow cavities with fat cells.

In April 1964 the patient informed us by letter that she was still suffering from slight dyspnea



Fig. 2. Photomicrograph of bony nodule. There re spicules of bone nd f t tissue resembling fat marrow.

Case 2

The patient wa married woman aged 65. For ver 10 years she had been suffering from hronic trophic rhinitis, dry cough and crust formation in the nose but no foul odour. Th crust disappeared during course f vitamin A. She had sl ght epista is for on month al d go, in connection with n acut febrile infection f irly large mount f blood nd lot ppeared in the mouth. This discharge lasted for two day and the sputum has since been blood mixed.

Examination showed that the blood count was n rmal and sedimentation rate 3 mm The nasal cavities were large with some crusts in the upper parts. The pharyn was ordi ary On coughing som bloody sputum wa brought up Roentgen xamination Pulmonary f brosis, shadows referable t foci, hilar shadows somewhat enlarged.

Bron hoscopic xamination December 1963 (V herl, Eino & Eero) The lary x was pparently ormal, the tracheal and bronchial mucosa slightly reddened I the lower part f the tra hea on the right wall, there was an area consisting of sessile od les. A few nodules were also seen laterally in the right main bronchus and in the posterior and inferior wall of the upper lobe branch, also

one protuberance in the lateral wall of the stem bronchus. Bloody mucus appeared at the opening of the right main bronchus and a small hemorrhage in the mucous membrane at the upper margin of the entrance to the upper lobe. A sample of mucus was taken for cytologic and bacteriologic study and for culture of tubercle bacilli and a nodule was removed from the stem bronchus for biopsy examination.

Bacteriologic examination revealed a gram-negative microorganism, streptococcus *a haemolyticus*, Neisseria. Culture of tubercle bacilli was negative.

Histological examination. No tumour suspect cells in sputum sample.

Cytological examination. The epithelium consisted in part of metaplastic cornified squamous epithelium. Bone tissue, loose connective tissue and fat tissue resembling fat marrow occurred subepithelially, but no cartilage.

Follow-up examination May 1966. The dry cough has persisted but not the hemoptysis. A crust appears occasionally in the nose. No dyspnea. Crust formation absent as long as vitamin A preparations are taken. Bacteriological examination of the nose showed a gram-negative organism. A chest film showed no shadows referable to foci.

COMMENT

Each of these patients had a typical tracheopathia osteoplastica associated with signs of chronic respiratory infection: in the first case a cured tuberculosis, in the second atrophic rhinitis and pulmonary fibrosis. Both came to bronchoscopy because of bloody expectorations, which did not occur later. In the first case, in which the nodules caused narrowing of the tracheal lumen, there was also slight dyspnea.

The cause of the condition is not known. Several hypotheses have been advanced as regards its etiology and pathogenesis. It has been regarded as multiple osteoma, exostosis, a congenital anomaly, or as originating from connective tissue elements. Most cases have the feature in common that they present signs of various chronic respiratory tract infections, e.g. bronchitis, sinusitis and ozaena. Jepsen & Sørensen call special attention to the fact that the histological changes in ozaena and in tracheopathia osteoplastica are much alike. Our cases, too, were associated with slight chronic infections. Histological examination revealed transformation of respiratory epithelium by metaplasia into a stratified squamous epithelium. On this basis the idea readily suggests itself that also the submucosal changes are attributable to chondro- and osteometaplasia of the connective tissue. Dalgaard has studied the histology and pathogenesis in great detail. Outside the bone lamellae he found elastic cartilage with gradual transition to elastic connective tissue. The same phenomenon appeared in our first case. This seems to bear out his statement that "the elastic cartilage arises directly from the elastic connective tissue through direct metaplasia. The cartilage may later calcify and ossify." Dalgaard (1947) found no association between the nodules containing elastic cartilage and the tracheal rings containing hyaline cartilage. In his cases the biopsy specimens were removed at autopsy.

The histological picture of tracheopathia osteoplastica also resembles the changes seen in keratosis pharyngis (Vaheri, 19 1). It is true that the epithelial changes differ in tracheopathia there is metaplasia and in keratosis an intense cornification, but each shows a metaplastic change of connective tissue into cartilage and bone. The basic cause of both these diseases is probably a chronic slowly advancing inflammatory process.

ZUSAMMENFASSUNG

Zwei Fälle on Tracheopathia osteoplastica wurden klinisch und histologisch u tersucht. Bei der Untersuchung wurde die Umwandlung des respiratorischen Epithels zum Plattenepith l festgestellt. Der sich in den Knoten befindlich elastische Kn rpel i t wahrscheinlich au h durch die metaplastische Veränderung aus d m lastischen Bindegew be entsta den. Der Knorpel kann später verkalken und verknöchern.

REFERENCES

Carr, D. T. and Olsen, A. M. 1954. Tracheopathia osteoplastica. *J.A.M.A.* 155, 1563.

Clerf, L. H., 1944. Tracheopathia osteoplastica. *Ann. Otol.* 53, 839.

D alla, J. 1947. Tracheopathia chondro-osteoplastica. *Act. Path. Microbiol. Scand.*, 24, 118.

Ebrva, K., 1964. Tracheopathia hondro-osteopl stica. *Nord. Med.* 72, 1029.

Hey ev, R., 1954. Tracheopathia osteoplastica. *Act. Otolaryng.* (Stockh.) 5 ppl. 116, 134.

Jackson, C., and Jackson, C. L., 1932. Benign tumors of trachea nd bronchi. *J.A.M.A.* 99, 1747.

J rsen, O., d Sörensen, H., 1960. Tracheopathi osteoplastica nd ozaena. *Act. Otolaryng.* (Stockh.) 51, 79.

Laurs, B., 1959. Dansk Otolaryng. Selsk. *Forhandl.* Quoted by Jepsen & Sörensen.

Vatthan, J. nd Cla s, H.-G., 1963. Z m endosk pischen nd röntgenologischen Bild der Tracheopathia chondro-osteoplastica. *Z. Laryng. Rhinol. Otol.*, 42, 452.

Morrison, H. J., Brooks, A. C., nd H vre, F. Z., 1937. Tracheopathia osteopla tica (osteoma of the trachea). *Arch. Otolaryng.* (Chic.) 26, 291.

P sov s, L. 1954. La trachéopathie hondro-ostéoplastique d point de vue clinique et pathogénique. *R. Laryng.* (Bord.) 75, 349.

Schmidt, W. 1949. Tracheopathia osteopla tica. *HNO* (Berlin) 1, 614.

V heri, Eino, 1931. K ratosis phary gis. *A t Otolaryng.* (Stockh.) S ppl. 15, 0.

Wilks, S., 1857. Ossific deposit on the lary x, trachea and bronchi. *Trans. Path. Soc. London* 8, 88. Quoted by Carr & Olsen.

Korvaklinikka,
Haartmaninkat 4, Helsinki 29,
Finland

Received December 10, 1964

Acta oto-laryngologica 64 256–262 (1967)

ACOUSTIC REFLEX AS A TEST OF INDIVIDUAL SUSCEPTIBILITY TO NOISE

A Preliminary Report

B. JOHANSSON, B. KYLIN and M. LANGFY
Stockholm, Sweden

From the Department of Technical Audiology, Karolinska Institutet, and the National Institute of Occupational Health, Stockholm

The "fatigue" of the acoustic reflex of the middle ear using pure tones and square wave-modulated pure tones is studied and compared with TTS for the same subjects after stimulation with broad-band noise. Close correlation has been shown between the TTS and the latency time, the rise time and the degree of the reflex for very short tone pulses.

Experience from the majority of studies on hearing of workers in noisy industries shows that there is a large individual difference in susceptibility to hearing damage from exposure to noise. A test has long been sought by which it would be possible to predict which persons may be considered to be susceptible and less susceptible (review by Ward, 1965 and 1967). Earlier attempts to find a relation between temporary changes in the hearing threshold after noise exposure and contralateral remote masking (CRM) test that provide a measure of the reflex contractions of the muscles of the middle ear have been unsuccessful. The contraction results in a change in sound transmission in the middle ear and should in some measure reflect the individual sensitivity to noise stimulus. However, the above experiments have been concerned chiefly with changes in threshold produced by noise with high frequency components. Later studies with exposure to low frequency noise (Cohen, Kylin & LaBenz, 1966) would seem to indicate that the indirect measure of the acoustic reflexes by the CRM test is correlated to temporary hearing threshold shifts after noise exposure, threshold shift being smaller for persons with a strong than a weak reflex.

In the study reported here a number of characteristics of the acoustic reflex have been examined with an acoustic impedance bridge by recording

Grants for this investigation have been provided by the Folksam Research Committee and the Grängesbergs Company Research on underground working conditions. Test subjects have been kindly made available by the Hospital of the Swedish Army School of Ordnance Administration.

changes in the acoustic impedance of the ear Some of these characteristics have been compared with the temporary threshold shift after exposure to low-frequency noise band (300–1200 Hz)

MATERIAL AND METHODS

A continuous registration of variations in the acoustic impedance of the ear is technically complicated and not possible with commercial instruments. Instead, an acoustic bridge was balanced for normal state and the absolute value of the change in impedance was recorded The acoustic reflex response—the muscular contractions of the middle ear—was determined by recording changes in in-impedance A commercial bridge intended for the purpose (Madsen Electronics, Model ZO 61) was modified and supplemented as shown in the block diagram in Fig 1

The pure tone generator in the original instrument (9) was replaced with an external generator (3) and a noise generator (1) with associated band pass filter (2) The two signal sources (1) and (3) were connected through a modulator gate unit (4) with trigger (5) and an attenuator (8)

The measurement frequency was altered from 220 Hz to about 750 Hz, as specified by Møller (1958) with a simultaneous change in the corresponding band-pass filter To obtain shorter time constants the band width of the filter was increased from 50 to 120 Hz.

To facilitate registration of contractions for a rapid sequence of the stimuli an amplifier-rectifier unit (10) was built with rise and decay times of 1 and 120 ms, respectively The registration was performed with an Elema Schönander Mingograph 24 B

In a preliminary run the acoustic reflex response to various types of stimulus was recorded on 50 subjects with normal hearing After an approximate determination f the reflex threshold for stimulus tones 500 and 2000 Hz, the reflex response of the muscles of the middle ear was examined with respect to contraction response to a stimulus lasting at least 20 sec at the above frequencies, with a sensation level of 110 dB The

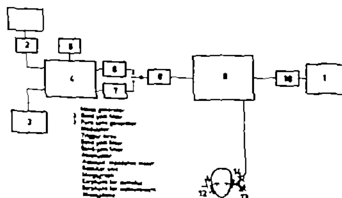


Fig 1

Acta oto-laryngologica 64 256–262 (1967)

ACOUSTIC REFLEX AS A TEST OF INDIVIDUAL SUSCEPTIBILITY TO NOISE

A Preliminary Report

B JOHANSSON B KYLIN and M LANGFY
Stockholm Sweden

From the Department of Technical Audiology Karolinska Institutet and the National Institute of Occupational Health Stockholm

The "fatigue of the acoustic reflex of the middle ear using pure tones and square wave-modulated pure tones is studied and compared with TTS for the same subjects after stimulation with broad-band noise Close correlation has been shown between the TTS and the latency time the rise time and the degree of the reflex for very short tone pulses.

Experience from the majority of studies on hearing of workers in noisy industries shows that there is a large individual difference in susceptibility to hearing damage from exposure to noise A test has long been sought by which it would be possible to predict which persons may be considered to be susceptible and less susceptible (review by Ward 1965 and 1967) Earlier attempts to find a relation between temporary changes in the hearing threshold after noise exposure and contralateral remote masking (CRM) test that provide a measure of the reflex contractions of the muscles of the middle ear have been unsuccessful The contraction results in a change in sound transmission in the middle ear and should in some measure reflect the individual sensitivity to noise stimulus However the above experiments have been concerned chiefly with changes in threshold produced by noise with high frequency components. Later studies with exposure to low frequency noise (Cohen, Kylin & LaBenz, 1966) would seem to indicate that the indirect measure of the acoustic reflexes by the CRM test is correlated to temporary hearing threshold shifts after noise exposure threshold shift being smaller for persons with a strong than a weak reflex

In the study reported here a number of characteristics of the acoustic reflex have been examined with an acoustic impedance bridge by recording

Grant f thi i estigati n ha e be n pro ided by the Folksam Rese rch Committee and the Gra gesbergs Company Research on u derground working cond ti s. Test bject ha e been ki dly m d a il bl by the Hospit l f th Swedi h Arm School f Ordn nce Administrati

changes in the acoustic impedance of the ear. Some of these characteristics have been compared with the temporary threshold shift after exposure to low-frequency noise band (300–1200 Hz).

MATERIAL AND METHODS

A continuous registration of variations in the acoustic impedance of the ear is technically complicated and not possible with commercial instruments. Instead, an acoustic bridge was balanced for normal state and the absolute value of the change in impedance was recorded. The acoustic reflex response—the muscular contractions of the middle ear—was determined by recording changes in in-impedance. A commercial bridge intended for the purpose (Madsen Electronics, Model ZO 61) was modified and supplemented as shown in the block diagram in Fig. 1.

The pure tone generator in the original instrument (9) was replaced with an external generator (3) and a noise generator (1) with associated band-pass filter (2). The two signal sources (1) and (3) were connected through a modulator gate unit (4) with trigger (5) and an attenuator (8).

The measurement frequency was altered from 220 Hz to about 750 Hz, as specified by Møller (1958) with a simultaneous change in the corresponding band pass filter. To obtain shorter time constants the band width of the filter was increased from 50 to 120 Hz.

To facilitate registration of contractions for a rapid sequence of the stimuli an amplifier-rectifier unit (10) was built with rise and decay times of 1 and 120 ms, respectively. The registration was performed with an Elema Schönander Mingograph 24 B.

In a preliminary run the acoustic reflex response to various types of stimulus was recorded on 50 subjects with normal hearing. After an approximate determination of the reflex threshold for stimulus tones 500 and 2000 Hz, the reflex response of the muscles of the middle ear was examined with respect to contraction response to a stimulus lasting at least 20 sec at the above frequencies, with a sensation level of 110 dB. The

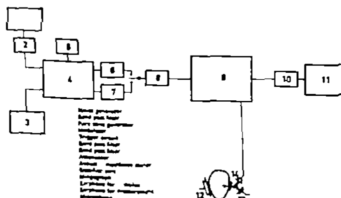


Fig. 1

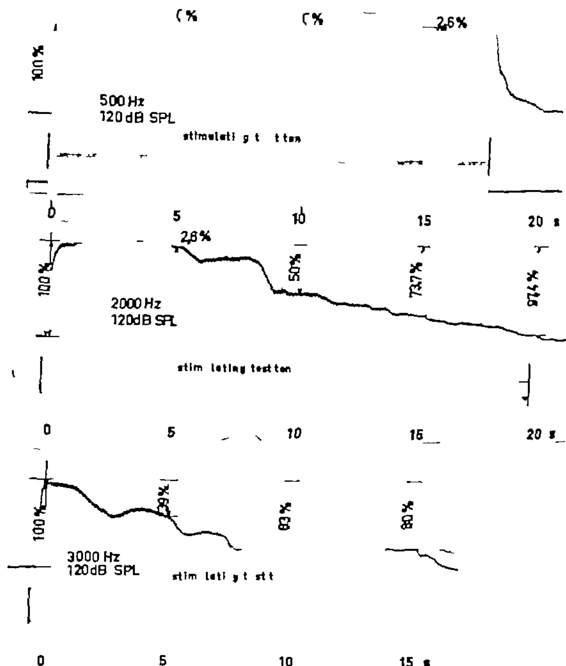
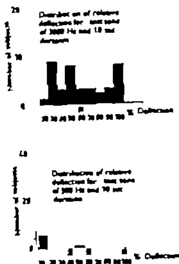


Fig. 2.

reflex response was then recorded during stimulus with a 3000 Hz tone which was impulse-amplitude modulated with 500 Hz by means of a multivibrator controlled gate During the modulation the impulse quotient was 50 per cent that is to say in any one cycle the signal was switched on half the time

On the basis of the results from the first test run 5 subjects were selected who were required to undergo a slightly modified examination of the acoustic reflex response The reflex response was then examined with respect to among other things, the latency and rise time for a stimulus at 1000 Hz and the form of the contraction curve for stimulation with short



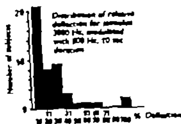


Fig. 3. Fig. 5.

pulses. The temporary threshold shifts after exposure for 15 minutes to a noise band of 300–1200 Hz, at a sound pressure level of 110 dB, was determined by recording the hearing threshold with a Békésy audiometer before and after exposure. After a hearing test including Békésy audiograms and the acoustic reflex response the subject was exposed to noise and new audiograms were begun 2 minutes after the exposure.

RESULTS

As is seen from Fig. 2, for 500 Hz the state of contraction of the muscles of the middle ear remained constant throughout the stimulus time while for the higher frequencies "fatigue" was observed fairly soon after the stimulus was begun. This fatigue was measured as the relative decline from full contraction after 10 seconds stimulus.

It is seen from the histogram in Fig. 3 that the distribution with respect to the deflection for the 3000 Hz stimulus would seem to be quite random

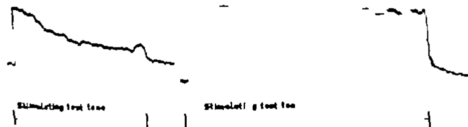


Fig. 4.

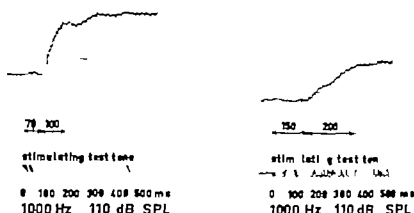


Fig. 6

For 500 Hz, on the other hand there was a markedly skew distribution, most of the subjects recording an extremely small decline

Fig 4 shows an example of the pronounced change in deflection when the 3000 Hz tone was impulse amplitude modulated with 500 Hz. Here too, the distribution of the subjects was skew but the proportion of extremely large deflections was higher than for the 500 Hz stimulus (Fig 5)

In an analysis of the relationship between the deflection and the temporary threshold shifts (TTS) after exposure to low frequency noise (300–1200 Hz) there was a tendency for the subjects recording an extremely large deflection to display also a large threshold shift for 1000 1500 and 2000 Hz. No such tendency was seen however for the group as a whole but only for the extreme subjects with "poor" acoustic reflex These results



Fig.

TABLE 1 *Temporary hearing threshold shifts in relation to the latency rise time and full activation time" of the acoustic reflex*

Subject	TTS (dB) 500 Hz	1000 Hz	Latency (ms)	Rise time (ms)	Percentage of full contraction t arlous tone pulses; 120 dB SPL t 1000 Hz 1000	250	100	50	20	10	5	2	1 ms
1	0	10	50	110	100	97	92	100	80	63	37	9	3
2	4	7	70	100	100	100	83	64	21	1	8	2	—
3	3	15	70	160	100	87	88	32	13	8	6	2	—
4	11	21	110	200	100	62	29	5	3	—	—	—	—
5	17	27	130	210	100	86	66	42	—	—	—	—	—

prompted a study of other characteristics of the acoustic reflex, namely the latency time, rise time and impedance change at a stimulus with 1000 Hz tone impulses from 1000 down to 1 ms in duration, and 120 dB SPL (Figs. 6 and 7)

The study was performed on five of the selected subjects who represented the extreme cases—that is, persons showing a large deflection and TTS and those with an insignificant deflection and small TTS.

As is seen from Table 1 there was a close correlation between the TTS, latency time, rise time and full acti ation time

DISCUSSION

"Fatigue in the acoustic reflex elicited by low frequencies, that is, the departure from full contraction during short duration stimulus with low frequency tones (500 Hz) wa extremely small for most of the subjects. Only a few showed a moderate deflection. For high-pitched tones, on the other hand there was a wide range of deflection in the series. This marked difference in acoustic reflex response to low and high frequency tones has recently been demonstrated by Djupesland, Flottorp & Winther (1967) (independently of the present study) and has earlier been reported by Anderson (1960)

High-frequency timulus produced fatigue in the reflex response al most immediately and there were large indi idual differences in this respect. Stimulus with low-pit hed tones, however appeared to be capable of m intaining the acousti reflex response without any appreciable deflection, nd the individual differences w r not so marked.

One explanation of th greater fatigue produced by the high pitched stimulu may be that the number of stimuli per unit of time for the reflex path for high-frequency tones exceeds th number permitted by the refractory period for the nerve path. S pport for thi speculation is found in the result of the experiment with th impulse-amplitude modulated

stimulus (3000 Hz/500 Hz) in which the reflex response was largely the same as for stimulus with 500 Hz, alone

The time function of the 3000 Hz signal in the form of 500 tone pulses per second can be represented by a line spectrum of 2500 3000 and 3500 Hz in the frequency function after the band pass filtration The ear also detects a tone with a level corresponding to a 500-Hz signal of the level in question The better reflex response obtained for this stimulus than for a continuous signal of 3000 Hz might be due to the interruptions in stimulation due to the impulse modulation That this is the case is indicated by the results of subsequent experiments (to be published)

The subjects with a poor acoustic reflex—that is, those who showed a large deflection in the reflex response to stimulus with an impulse modulated tone—also recorded a large temporary threshold shift TTS, after exposure to noise Moreover a close correlation was found between the TTS and the latency time of the acoustic reflex its rise time and its full activation time

The results prompted a closer examination of suitable tests for noise susceptibility by using one or more parameters for the acoustic reflex A study has also been made of the possible connection between permanent hearing damage and the acoustic reflex (Johansson Kylin & Langfy to be published)

ZUSAMMENFASSUNG

An einer Gruppe von 60 Personen wurde das Abklingen („fatigue") des akustischen Mittelohrreflexes mit sin Tönen und rechteckmodulierten sin Tönen untersucht und an 5 von diesen Personen mit dem TTS (temporary threshold shift) nach Beschallung mit Breitbandgeräusch verglichen Es zeigte sich des weiteren eine gute Korrelation zwischen dem TTS und der Latenzzeit Anstiegszeit und Stärke des Reflexes für sehr kurze Tonimpulse.

REFERENCES

Anderson H 1966 P rsonal communication

Cohen, A Kylin B and LaBenz, P 1966: T mporary thresh ld shift in heari g from exposure to combined impact st ad t t ol conditio *J A S A*

Djupesland, G Flottorp G and Winther, E Q 1967 Size d duration of acoustic lly licited impedance changes in m n Pres nted at th 16th Congress of Scandina ian Oto-laryngical Society J n 27–30 1966 *Acta Otolaryng* (Stockh) In press.

Møller, A 1958 I tra-aural muscle co tracti n i man, xami ed by measuring acou ti mpedance of the ear *Laryngoscope 68* 48

Ward, W D 1965 Th concept of sceptibility t he ri g loss *J Occ Med 12* 595.

— 1967 Susceptibility t udit ry fatigu [*Preprint*] In *S nsory Ihy iology* (ed. by L. D N ff) A d mic Press, New York.

B J hansson Th D pt f Techn cal Audiology Karolinska Inst tut t Stockholm 60 Sweden

R cei ed F bruary 7 1967

Acta oto-laryngologica 64, 263–266 (1967)

VIRUS-LIKE PARTICLES IN HUMAN ORAL PAPILLOMA

L. FRITHIOF and J. WERSÄLL
Stockholm, Sweden

From King Gustaf V Research Institute and the Department of Otolaryngology, Karolinska Sjukhuset and the Department of Oral Surgery, Odontological Faculty, Karolinska Institutet, Stockholm

Virus-like particles were found in nuclei of stratum corneum cells in one case of human lingual papilloma studied by electron microscopy. The size and structure of the particles resembled the papilloma viruses as described in the literature. Filaments with a characteristic structure and small irregularly-shaped particles were found in other nuclei where virus-like particles were absent. It is proposed that the filaments and the irregular particles represent stages in viral transformation.

Papillomatosis of the oral cavity in the dog (Cheville & Olson, 1964; De Monbreun & Goodpasture, 1932) and papillomas in some other species (Rdzok, Shipkowitz & Richter, 1966; Shope, 1962; Stone, Shope & Moore, 1959; Williams, Kass & Knight, 1960) have been shown to be of viral origin. Most of the human papillomas are also considered to have a viral etiology (Jarvi, 1944; Ullmann, 1922) and the ultrastructure of cells and viruses has been described in a number of papers (Almeida, Howatson & Williams, 1962; Bunting, 1953; Charles, 1960; King & Finch, 1964; Strauss *et al.*, 1949; Williams, Howatson & Almeida, 1961).

Little information is available, however, on the human, oral papilloma (Shafer, Hine & Levy, 1962) and we have been unable to find any report confirming the theory of a viral etiology. Descriptions of virus particles in human oral papillomas are rare (Fasske, Morgenroth & Themann, 1964).

In view of this, the present report on the finding of virus-like particles and certain cellular changes in the epithelium of a human lingual papilloma may be of interest. Thin sections from the osmium fixed and Epon embedded (Frithiof, Lagerlöf & Wersäll, 1964) tissue were stained in uranyl acetate and lead acetate solutions. The following observations were made in a Siemens Elmiskop I.

The basal and spinous cells were hypertrophic, with the cell organelles widely scattered within the cytoplasm. The structure of the nuclei was vacuolated and less dense than in normal epithelium (Frithiof, Lagerlöf & Wersäll, 1964). The number of desmosomes increased considerably only a few cell layers above the basement membrane, as the cells assumed a flattened shape.

This work has been supported by the Swedish Cancer Society and the Odontological Faculty, Karolinska Institutet.

Normally when a stratum corneum cell is formed there is a sudden change of structure involving the whole cell (Frithiof Lagerlof & Wersall 1964)

The first signs of keratin transforming were observed here as a concentration of dense material in the distal part of cells close to the hyperparakeratotic stratum corneum

The cytoplasm of most stratum corneum cells had an unusual flocculated appearance

Within the lowest part of the *stratum corneum, ribosomes could* still be observed forming small clusters

In the central and the superficial part of the stratum corneum *several* nuclei contained numerous oval or round particles They had a fairly *constant diameter of 350 Å* and a varying electron density Particles with low density often showed a ring shaped structure with a less dense central area Most particles, however had a central core of irregularly packed, dense granules and were surrounded by a single layer of extremely fine granules regularly spaced at a distance of only about 40 Å from the central core Outside this granular coat a less dense halo (peripheral coat) about 80 Å thick could be observed Particles, partly situated within the extremely thick layer of marginated chromatin were seen without a halo Particles were not *found in the intercellular space* but could be seen in the cytoplasm of a few cells

In a few nuclei where the particles were extremely dense and had an irregular shape, a complex fibrillar material was seen which had a fine transverse banding The fibrils, which also were seen in the adjacent cytoplasm had a diameter of 140 Å This characteristic fibrillar material might represent a stage in viral transformation It has been indicated by experimental studies (Shope 1962) that by transformations, viruses also exist in a shape which is not recognizable by means of ordinary electron *microscopic methods* (Ahlström 1965)

It is probable that the hypertrophic basal cells as well as the anomalous *cytoplasmic and nuclear structure within the basal and central* epithelium are due to the influence of this form of unvirus-like viruses (Shope 1962)

A granulated linear structure has been observed in extracted and negatively stained preparations of Shope papilloma viruses (Williams, Kass & Knight 1960)

Excluding the granular coat" which is only seen at high resolution the *particles in question have* several features in common with other papilloma viruses, which they resemble by their size granular central core less *dense halo intranuclear occurrence and* distribution within the keratinized part of the epithelium The abnormal cellular and nuclear morphology of the basal and spinous cells has been observed also in other papillomas In this specimen however particles have not been observed in crystalline aggregates (Almeida Howatson & Williams, 1962 Rdzok Shipkowitz & Richter 1966 Strauss *et al* 1949) and they are not distri-

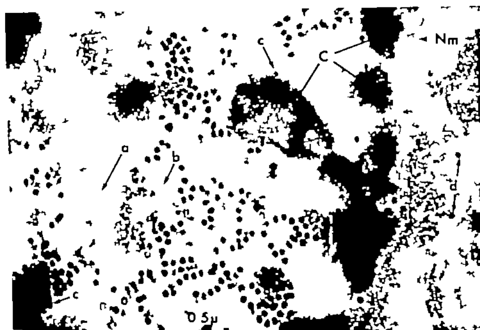


Fig. 1 Part f nucleus w th thick l yer f hromat (C lose t the lear membra (Nm) Numerous irus-l ke particle re seen in the ucleus (a–c) nd few i the cytoplasm (d) ( ) Particle with hal or peripheral coat, (b Ring-shaped particle Particles partly situated withi the chromatin d not b halo. 51,000

buted in, or connected by a reticular network (Almeida, Howatson & Williams, 1962 Cheville & Olson 1964 St ne Shope & Moore 1959)

The unu ually firm attachment between neighbouring cells with an increased number of desmosomes might ha e some importance in view of background t the macroscopic appearance of the p pilloma.

It should be empha ized that the definite establishment of a virus as an etiological agent in a disease mu t also include experimental, biological methods.

ZUSAMMENFASSUNG

Virusähnliche P rtikeln wurden im Kern der Strat m-corneum-Zellen in inem Fall von Zungenpapillom beim Menschen gefunden u d mit Hilf ines Elektronenmikroskops studiert. Sie und Strukt der Partik ln ähn lt dem papillomen Virus, wie es in der Literat r beschrieben ist. Fasern m t einer harakteristischen Struktur und kleinen, regelmä sig gemusterten Partikeln wurden in anderen Kernen gef nden w di irusäh lichen Partik ln ieht vorhanden w ren. E w rd angenommen, da die F sern und di unregelmäsigen Par t k ln St fen in der viralen Tra sformat on d rstellen

REFERENCES

AHLSTRÖM, C. G. 1965: Do viruses play any role in human cancer? *Unio Nordica Contra Cancrum. The Swedish Cancer Soc. Symp. on Virus and Cancer. Saltsjöbaden, 1965* (eds. H. Bergstrand and K. E. Hellström). Stockholm, p. 7.

ALMEIDA, J. D., HOWATSON, A. F. and WILLIAMS, M. G. 1962: Electron Microscope Study of Human Warts. Sites of Virus Production and Nature of the Inclusion Bodies. *J. Invest. Dermat. 38*, 337.

BUNTING, H. 1953: Close Packed Array of Virus Like Particles within Cells of a Human Skin Papilloma. *Proc. Soc. Exp. Biol. Med., 84*, 327.

CHARLES, A. 1960: Electron Microscope Observation on the Human Wart. *Dermatologica 121*, 193.

CHEVILLE, N. F. and OLSON, C. 1964: Cytology of the Canine Oral Papilloma. *Amer. J. Path. 45*, 849.

DEMONBREUN, W. A. and GOODPASTURE, E. W. 1932: Infectious Oral Papillomatosis of Dogs. *Amer. J. Path. 8*, 43.

FASSKE, E., MORGENROTH, K. and THEMANN, H. 1964: Elektronenoptischer Virusnachweis im menschlichen Mundschleimhautpapillom. *Klin. Wochnschr. 42*, 546.

FRITHIOF, L., LAGERLÖF, B. and WERSÄLL, J. 1964: Electron Microscopical Observations on Hyperkeratinization in Oral Mucosa. *Acta Otolaryng.* (Stockh.) Suppl. *188*, 57-423.

JARVI, O. 1944: Kieferhöhlpapillom mit (virusbedingten?) Cytoplasmaeinschlüssen. *Acta Otolaryng.* (Stockh.) *32*, 284.

KLUG, A. and FINCH, J. T. 1964: Structure of Viruses of the Papilloma-Polyoma Type. I. Human Wart Virus. *J. Mol. Biol. 11*, 403.

RDZOK, E. J., SHIPKOWITZ, N. L. and RICHTER, W. R. 1966: Rabbit Oral Papillomatosis: Ultrastructure of Experimental Infection. *Cancer Res. 26*, 160.

SHAFER, W. G., HINE, M. K. and LEVY, B. M. 1963: *A Textbook of Oral Pathology*. 2nd ed. W. B. Saunders Co. Philadelphia and London.

SHOPE, R. E. 1962: Are Animal Tumor Viruses Always Virus-Like? *J. Gen. Physiol. Suppl. 5*, 143.

STONE, R. S., SHOPE, R. E. and MOORE, D. H. 1959: Electron Microscope Study of the Development of the Papilloma Virus in the Skin of the Rabbit. *J. Exp. Med. 110*, 543.

STRAUSS, M. J., SHAW, E. W., BUNTING, H. and MELNICK, J. L. 1949: "Crystalline" Virus-Like Particles from Skin Papillomas Characterized by Intranuclear Inclusion Bodies. *Proc. Soc. Exp. Biol. Med. 72*, 46.

ULLMANN, E. V. 1922: Ätiologie und Pathogenese des Larynxpapilloms. *Z. Ohrenheilk. 3*, 514.

WILLIAMS, R. C., KASS, S. J. and KNIGHT, C. A. 1960: Structure of Shope Papilloma Virus Particles. *Virology 12*, 48.

WILLIAMS, M. G., HOWATSON, A. F. and ALMEIDA, J. D. 1961: Morphological Characterization of the Virus of the Human Common Wart (Verruca Vulgaris). *Nature 189*, 895.

L. Frithiof, M.D., Dept. of Oral Surgery, Odontological Faculty, Karolinska Institutet, Stockholm 60, Sweden

J. Wersäll, M.D., Dept. of Otolaryngology, Karolinska Sjukhuset, Stockholm 60, Sweden

Received December 1, 1966

Acta oto-laryngologica 64 267 279 (1967)

HISTOCHEMICAL STUDY OF THE ACETYLCHOLINESTERASE ACTIVITY IN THE INNER EAR OF THE SQUIRREL MONKEY

T Ishii Y Murakami[3] and R R. Gacek

Boston Mass USA

From the Departm t f Otol ryngology (Head Prof H F Schukne ht) Massachusetts Ey and Ear Infirmary and H rvard Medical School, Boston

Perfusion-fi ti n was performed the squirrel monkey whose temporal bones were then decalcified in cold EDTA (thylenediamine tetraacetat) solution Cholinesterase (ChE) activity was demonstrated on frozen secti of these bones. ChE in th fferent fibers f the inner ea was identified as cetylch linesterase (AChE) by using specific inhibitors. Fin scatt red AChE containing fibers in th cochlear nerve were bserved crossing the glial Schwan sheath junction which were ot a part of Rasmussen bundle After transection of th cochlear erv these fin fibers lost AChE cti ity suggesting they were of efferent nature In the organ of Corti, both the upper t nn l radial and basilar bundles were found t show AChL acti ity These tw bundles apparently contained efferent fibers.

INTRODUCTION

The efferent innervation to the inner ear was first studied by the neuroanatomical approach (Rasmussen 1946)

Acetylcholinesterase (AChE) was first demonstrated in the organ of Corti of the cat by Churchill Schuknecht & Doran (1956) and this AChE cti ity disappeared following sectioning of the olivo-cochlear bundle (Schuknecht, Churchill & Doran, 1959 Gacek Nomura & Balogh, 1965) Th efferent fiber component in the vestibular system also was demon

This study w supported by research grant NB 04153-04 and NB 03532-05 from the National Institutes of Neurological Diseases and Blindness, N tion l I titutes f Health of the U ited St tes Public Health Service

Formerly Rese rch Fellow Mass. Ey nd Ea I firmary and Harvard Medical School. Now Assist nt in the Department of Otorhinol ry gology Faculty of Medicine U i ersity of Toky

Research F llow Ma s. Ey nd Ea Infirmary nd Harv rd Medical School.

Assistant in the Department f Ot lary gology Mass. Ey nd Ear Infirmary nd Harvard Medical School.

REFERENCES

AHLSTRÖM, C. G. 1965: Do viruses play any role in human cancer? Uni Nordica Contr Cancrum Th Swedish Cancer Soc Symp on Virus and Cancer Saltsjöbaden, 1961 (eds H Bergstrand and K. E. Hellström) Stockholm, p. 7

ALMEIDA, J D HOWATSON A F and WILLIAMS, M G 1962: Electron Microscope Stud of Human W rts Sites of Virus Production and Nature of the In lusion Bodies. *J Invest Dermat 38* 337

BUNTING, H 1953 Close Packed Array of Virus-Lik Particles within Cells f a Human Skin Papill ma *Proc Soc Exp Biol Med 84* 327

CHARLES, A 1960 Electron Microscope Observations on the Human Wart *Dermatologica 121* 193

CHEVILLE, N F and OLSON, C., 1964: Cyt logy of the Canine Oral Papill ma. *Amer J Path* 45 849

DEMONBREUN W A and GOODPASTURE, E W 1932: Infecti us Oral Papillomatosis of Dog *Am J Path 8* 43

FABSKE, E., MORGE ROTH, K and THEM NN H 1964 Elektronen ptischer Virusnachweis im menschlich n Mundschleimhautpapill m *Klin Wochenschr 42* 546.

FRITHIOF L LAGERLÖF B nd WERSÄLL, J 1964: Electron Microscopic I Observations on Hyperkeratinization in Oral Mucosa *Acta Ot laryng* (Stockh) Suppl *188* 87 422.

JANVL, O 1944: Kieferhöhl npapillom mit (iru bedingten?) Cyt pl maeinschlüssen. *Acta Ot laryng* (Stockh) *32* 284

KLUG, A and FINCH, J T 1964 Structure f Viruses of the Papill ma Poly ma Type. I Human Wart Virus. *J M l Bi l 11* 403

RDZOK, E. J SHIPKOWITZ, N L., and RICHTER, W R 1966 Rabbit Oral P pillomatosi : Ultrastructure of Experim nt l Infecti n *Cancer R s 26* 160

SHAFER, W G HINE, M K and LEVY B M 1962 *A T xtbook f Oral Pathology* 2nd ed W B. Saunders Co Philad lphia and London.

SHOPE, R E., 1962 Are Animal Tum r Viruses Alway Virus-Lik ? *J Gen Physiol* Suppl 45 143

STONE, R S SHOPE, R E., and MOORE, D H 1959 Electron Microscope Study of the Dev l pm nt of the P pilloma Viru in the Ski of the Rabbit. *J Exp Med., 110* 543

STRAUSS, M J SHAW E. W BUNTING, H nd MELNICK, J L., 1949 "Crystallin Virus-Lik Parti les f m Skin Papill m s Characterized b I tra n lea Incl ion Bodies. *Proc Soc Exp. Bi l Med 72* 46

ULLM N E. V 1922: Ati logie und Pathogenese d Lary papill ms. *Z Ohrenheilk 3* 514

WILLIAMS, R C., KA S, S J nd KN HT C. A 1960 Stru t re f Shope Papill ma Virus P rticles *Virology 12* 48

WILLI MS, M G How TSON A F n d ALMEIDA, J D 1961 Morph logical Characteriz ti n f th Viru f th Hum n Comm n W rt (V rruca Vulg ris) *Nature 189* 895

L. Frith f M.D Dept of Oral S rgery Od ntolog cal Fa ulty Karolinsk In titutet Stockh lm 60 Swed n

J W rsäll M.D Dept f Ot laryngology Karolinska Sjukhu t Stockh lm 60 Swed n

Received December 1 1966



Fig. 1 AChE act lty f the tensor tympa i muscl included withi the t mporal bone section. The neuromuscula end-pl tes show adequ t ly localized histochemical reac tio 500.



F 2 Frozen sectio of the brain stem nd temporal bone of the normal monkey (Chl acti y i seen i some part of the brai tem, estibular efferent and stra g nglion p l bundles 17

Tran ection of the Cochlea Nerve

Tw monkeys were used for this periment Under anesthesia with pentoba bital injection, the posterior fossa craniotomy wa performed. Then, the cerebellum wa r tracted out of the cerebello-pontine angle to

strated neuroanatomically (Rasmussen & Gacek 1958) and histochemically (Dohlman Farkashidy & Salonna 1958)

Subsequent histochemical studies revealed that AChE activity was present in nerve fibers in the osseous spiral lamina intraganglionic spiral bundles and vestibulo-cochlear anastomosis (Del Bo & Conti, 1961 Rossi, 1961 Wersäll Hilding & Lundquist 1961 Hilding & Wersäll 1962 Smith & Rasmussen 1963 Gacek Nomura & Balogh 1965 Rossi & Cortesina 1966) and in the vestibular system (Ireland & Farkashidy 1961 Rossi, 1961 Hilding & Wersäll 1962 Rossi & Cortesina 1963 1965 and 1966 Nomura Gacek & Balogh, 1965) Histochemical studies of the human efferent fibers were reported by Nomura & Schuknecht (1965) and Ishii, Murakami & Balogh (1967)

In the present paper we employed perfusion fixation of the squirrel monkey to improve AChE localization and morphological preservation Some new features of the efferent system in the inner ear were also demonstrated

MATERIAL AND METHODS

Preparation of Unoperated Animals

Animals used here were three healthy adult squirrel monkeys Under anesthesia by pentobarbital injection blood was washed out through the ascending aorta with Ringer solution then the animals were perfused with ice-cold neutral formol-calcium solution for 15 minutes. Temporal bones were removed with brain stem attached allowed to be fixed in the same fixative overnight (Ishii Murakami & Balogh 1967) Fixed specimens were decalcified in EDTA solution at 4 C for 2 weeks (Balogh & Nomura, 1964)

Decalcified tissue blocks were frozen on dry ice and sectioned serially at 15 μ in a cryostat Horizontal and vertical sections were obtained Then sections were thawed dried on the coverslips and incubated in a substrate solution at 37 C for 3 hours The substrate solution was prepared according to Koelle's method modified by Gomori (1952)

After incubation, the sections were washed in 3 changes of saturated sodium sulfate, treated with diluted ammonium sulfide solution and dehydrated in graded alcohol Finally they were cleaned with xylene and mounted in Canada balsam previously saturated with copper sulfide

Inhibitory tests were carried out to specify cholinesterase activity (Pepler & Pearse 1957) eserine salicylate solution (in a final concentration 10^{-4} M) as an inhibitor for AChE (specific ChE or true ChE) and ChE (non specific ChE or pseudo-ChE) Silver nitrate (10^{-3} to 10^{-4} M) and iso-OMPA (tetra isopropylpyrophosphoramide 10^{-3} to 10^{-6} M) were used before and during incubation for the inhibition of non specific ChE

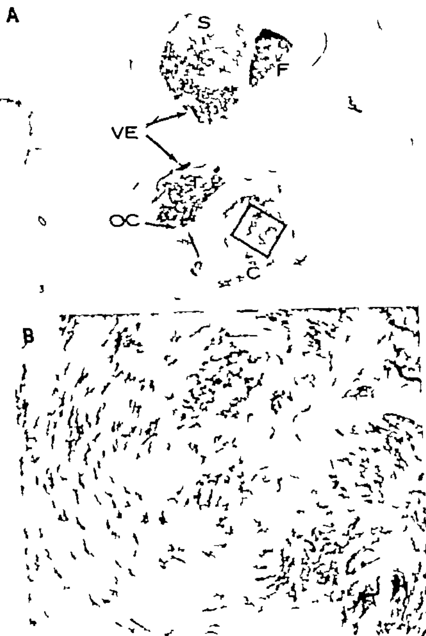


Fig. 4. Cross section of the internal auditory canal in the normal monkey. (A) Intense activity is observed in the vestibular efferents (VE) and the olivo-cochlear bundle (OC). Scarpa's ganglion cells are negative for AChE. The squared area is magnified in B. F, facial nerve; S, superior division of the vestibular nerve; I, inferior division of the vestibular nerve; C, cochlear nerve. ×28. (B) Higher magnification of a part of the cochlear nerve. Fine AChE containing fibers are distributed throughout the nerve. They are not a part of the olivo-cochlear bundle. ×220.

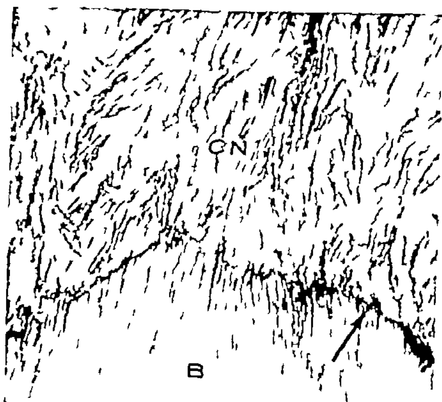


FIG. 3 Enlarged ph tomicrograph of the cochl ar nerve root. Numerous fin AChE containing fibers cross the glial Schwann sheath junction B brain st m CN cochlear n rve Arrow points to the glial-Schwann sh ath junction. × 220

expose the nerve The cochlear and vestibular nerves were identified as they course to the internal auditory meatus The cochlear and vestibular nerves were easily separated by blunt dissection Using the right angle knife, the cochlear nerve was transected and a piece of gel foam was in serted between the cut ends of the cochlear nerve.

The operated animals were allowed to survive for 1 and 3 weeks Then, they were sacrificed and specimens were prepared as described above The ears of the unoperated side were used as control

RESULTS

AChE activity was demonstrated as a distinct brown precipitate Histochemical reaction for AChE and ChE was inhibited by eserine salicylate (10^{-4} M) throughout the cochlea Silver nitrate (10^{-3} M) and iso-OMPA (10^{-3} M) failed to inhibit the histochemical reaction in the efferent fibers, indicating they were exclusively AChE

Even after 2 weeks decalcification in EDTA perfusion fixation gave a satisfactorily distinct localization Fig 1 illustrates the neuromuscular end plates of the tensor tympani muscle in the same temporal bone sections, presenting a minimal diffusion of the enzyme

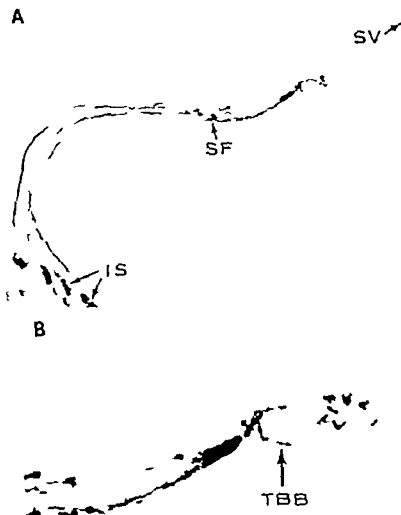


Fig. 6. Frozen section of the normal cochlea. (A) AChE activity is seen [illegible] the intra ganglionic spiral bundles (IS) and the spiral f[illegible] in the osseous spiral lamina SF. The spiral ganglion cells and the stria [illegible] scalaris (SV) show [illegible] response t[illegible] the enzyme activity. ×30. (B) Higher magnification of the organ of Corti from (A). The t[illegible] basilar bundle (TBB) shows AChE activity [illegible] the upper t[illegible] radial bundle does. The nerve endings and the cochlea [illegible] show AChE activity. ×390.

A transverse section of the nerves in the internal auditory canal and associated end organs [illegible] shown in Fig. [illegible] (A). Fine AChE-active efferent fibers are distributed throughout the peripheral vestibular organs (Fig. 7 (A)). Fig. 7 (B) demonstrates terminal portions of the efferent fibers to the macula utriculi and sacculi.

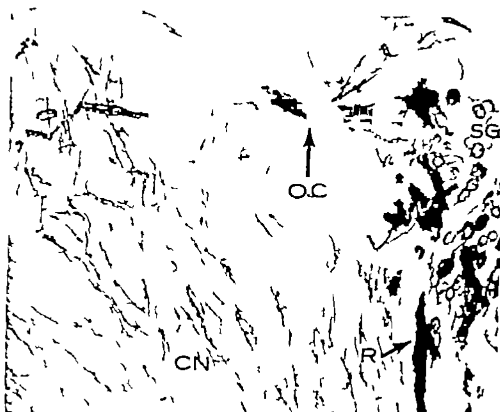


Fig 5 H rizontal section f the bottom of the intern l audit ry canal AChE ctl it is demo strated in th olivo-cochl ar bu dle (*OC*) nd Ra mussen bu dle (*R*) Fine AChE containing fibers are also seen in th cochl ar nerve (*CN*) The saccul r ganglion cell (*SG*) are negati fo AChE. × 150

Normal Ears

A horizontal section through the inner ear and adjacent brain stem is seen in Fig 2 The vestibular efferent and intraganglionic spiral bundles were stained dark At a higher magnification of the cochlear nerve root, numerous AChE containing fibers were seen traversing the glial Schwann sheath junction (Rasmussen, 1946) (Fig 3) A cross section of the nerves in the internal auditory canal also revealed the vestibular efferent fibers and olivo-cochlear bundle with high AChE activity (Fig 4 (A)) The enlarged photomicrograph of the cochlear nerve in Fig 4 (B) demonstrated a number of small caliber fibers containing AChE distributed throughout the cochlear nerve

Intense AChE activity was demonstrated in Rasmussen s bundle and the olivo-cochlear bundle while Scarpa s ganglion cells remained negative for AChF activity (Fig 5)

The intraganglionic spiral bundles and spiral fascicles in the osseous spiral lamina showed intense activity whereas spiral ganglion cells and stria vascularis failed to react (Fig 6 (A)) In the organ of Corti the internal spiral bundle and three outer spiral bundles showed high enzyme activity Both upper tunnel radial and basilar tunnel bundles were AChE-positive (Fig 6 (B))

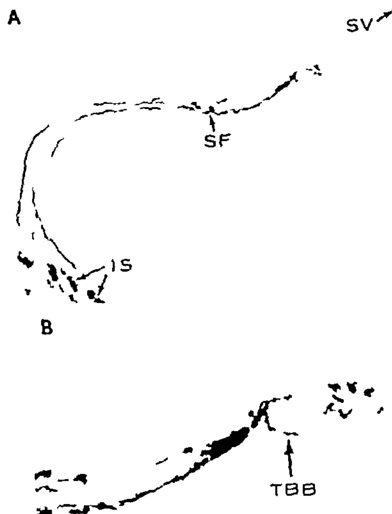


FIG. 4. Frozen section f the ormal cochlea. (A) AChE acti ity is seen i the intra g nglionic spiral bundles (*IS*) and the spir l f scicles in the osseous spiral lamina SF The *piral ga glion cells* nd the stria ascularis (*SV*) how no response t the enzyme acti ity 120 (B) Higher magnific tion of the orga f Corti from (A) The t nel ba il bundle (*TBB* show *AChE acti ity* well as the pper tunnel radial bundle does. The nerv endi gs nd the cochlea plexus show AChE acti ity 350.

A tran erse section of the nerves in the internal auditory canal and a sociated endorgans i shown in Fig (A) Fine, AChE-active efferent fibers are di tributed throughout the peripheral vestibular organs (Fig 7 (A)) Fig. 7 (B) demon trates terminal portion of the efferent fibers i the macula utriculi and sacculi.

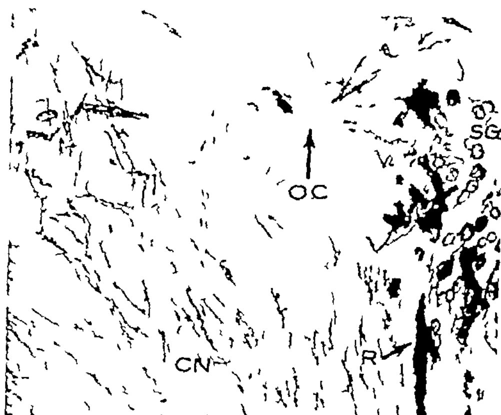


Fig 5 H l ontal secti n of th bottom of the internal audit ry nal AChE acti it is dem nstrated in the olivo-cochlear bundl (OC) and Ra mussen s bu dle (R) Fin AChF containi g fibers are lso een in the cochl a nerve (CN) Th saccular ganglion c ll (SG) re n g tive for AChE ×150

Normal Ears

A horizontal section through the inner ear and adjacent brain stem is seen in Fig 2 The vestibular efferent and intraganglionic spiral bundles were stained dark At a higher magnification of the cochlear nerve root numerous AChE containing fibers were seen traversing the glial Schwann sheath junction (Rasmussen, 1946) (Fig 3) A cross section of the nerves in the internal auditory canal also revealed the vestibular efferent fibers and olivo-cochlear bundle with high AChE activity (Fig 4 (A)) The enlarged photomicrograph of the cochlear nerve in Fig 4 (B) demonstrated a number of small caliber fibers containing AChE distributed throughout the cochlear nerve

Intense AChE activity was demonstrated in Rasmussen s bundle and the olivo-cochlear bundle while Scarpa s ganglion cells remained negative for AChE activity (Fig 5)

The intraganglionic spiral bundles and spiral fascicles in the osseous spiral lamina showed intense activity whereas spiral ganglion cells and stria vascularis failed to react (Fig 6 (A)) In the organ of Corti the internal spiral bundle and three outer spiral bundles showed high enzyme activity Both upper tunnel radial and basilar tunnel bundles were AChE positive (Fig 6 (B))

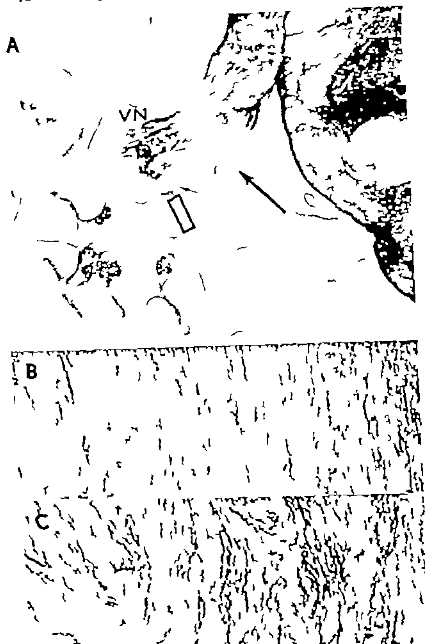


FIG. 8 Frozen section of the monkey sacrificed 1 week after transection of the cochlear nerve. A Photomicrograph showing complete transection (arrow) of the cochlear nerve caudal to the vestibular nerve (VN). The squared area of the cochlear nerve was shown in higher magnification in (B) × 17. (B) High magnification of the area indicated in (A) showing loss of activity in the cochlear nerve trunk. Small granules of activity represent the debris of degenerating fibers after 1 week following transection. Compare to (C) × 200. (C) Comparable area in the cochlear nerve of the unoperated control side of the same animal, showing normal activity of the fibers in the cochlear nerve trunk. × 200.

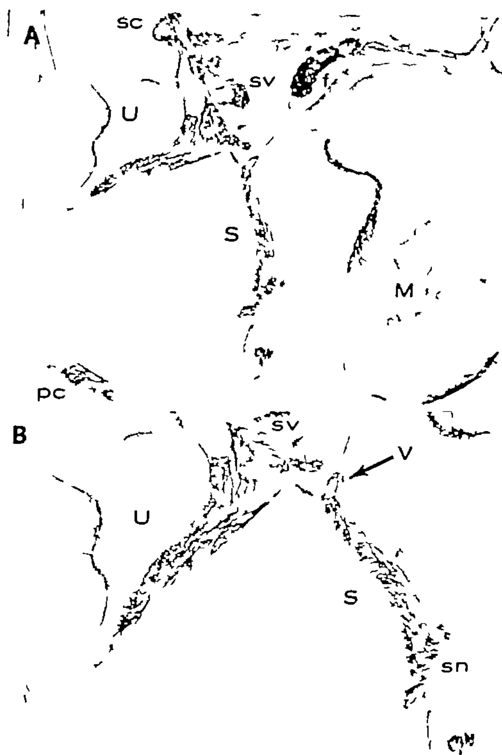


Fig 7 V rt l secti t th l g l of th l t rn l nd t rv l (A) Th peripheral estibul ff re t f bers re h w l th rist mp ll peri r and posterio (pc d macul tricull (U) nd sacculi (S) M modi lu f f ial nerv p s perio d isi f th estibul erv 25 (B) Highe m gnification f the nt icl (U) d saccul (S from th secti n bo P iph ral effere t fibers d nerve ndings are bserved. V V t bu dle ar peri di l l f the tibular n rve sn saccular nerve 39

ChE was histochemically demonstrated in the fibrous astrocytes, other neuroglia and capillary wall whereas AChE was present in the perikaryon, axon and dendrites of neuron (Koelle 1954 and 1955 Adams 1965) The specific inhibitory capability of iso-OMPA has been confirmed biochemically (Aldridge 1953 Austin & Berry 1953) A histochemical study (Pepler & Pearse, 1957) revealed that eserine inhibited both AChE and ChE, while only non-specific ChE was inhibited by silver nitrate and iso-OMPA. The cholinesterase in the efferent fibers of the inner ear was identified as AChE in the present study

In a previous report (Ishii, Murakami & Balogh 196) the fine caliber fibers containing AChE were described scattered throughout the human cochlear nerve Because of their distribution they do not appear to be part of the olivo-cochlear bundle of Rasmussen

The cochlear nerve of the squirrel monkey is anatomically similar to that of man Scattered fine caliber fibers demonstrating high AChE activity were also found in normal cochlear nerves of the squirrel monkey The efferent nature of these fibers was demonstrated by the loss of this activity following careful transection of the cochlear nerve near the brain stem This loss of activity was rapid correlating well with the rate of Wallerian degeneration in axon severed from their cell bodies. The results of this type of lesion locates the cell bodies of these efferent fibers generally in the brain stem but not specifically within any nucleus. However the cochlear nucleus could be a likely site

In the squirrel monkey this system of efferent fibers appears to consist of large number of very small caliber (less than 1 μ) fibers which arise central to the glial Schwann sheath junction of the cochlear nerve traverse this zone directly and are distributed uniformly between the afferent axons in the peripheral portion of the cochlear nerve Although these could be followed out as far as the spiral ganglion, their precise termination could not be determined

It is possible that there is a species difference in the size of this efferent system It appears to be much fewer in number in the cat cochlear nerve and almost non-existent in the guinea pig cochlear nerve (unpublished data) The significance of this efferent fiber system is not clear

In this study the localization of high AChE activity along the classical course of the olivo-cochlear efferent bundle was confirmed However there was a difference in AChE localization within the organ of Corti Both the upper tunnel radial bundle and the basilar radial bundle were seen to contain high AChE activity in the squirrel monkey It is reasonable to presume that both of these tunnel bundles contain efferent fibers in the monkey But, based on the electronmicroscopic findings after transection of the cat olivo-cochlear bundle Spoendlin & Gacek (1963) concluded that efferents exist only in the upper tunnel radial bundle while the basilar bundle is afferent. The discrepancy of this point remain to be determined

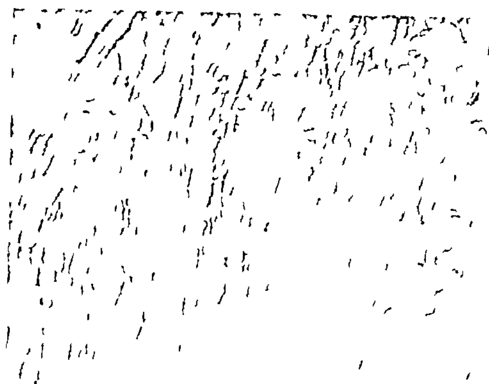


Fig 9 Cochlear nerve 3 weeks fter following transection howing complete l s of activity \ te loss of granules of acti ity found in more acute lesio Compare with *Fig 8 B.* × 200

Transection of Cochlear \erve

A section through the ear on the operated side of a monkey sacrificed 1 week after transection of the cochlear nerve is seen in Fig 8 (A) AChE activity was normal in the vestibular efferent fibers and intraganglionic spiral bundles indicating the olivo-cochlear bundle was intact The fine fibers containing AChE were diminished markedly throughout the cochlear nerve distal to the point of transection

After 3 weeks following the transection of the cochlear nerve complete loss of AChE activity was observed (Fig 9)

DISCI SSIO\

Fixation before incubation minimized the diffusion of AChF (Ravin, Tsou & Seligman 1951 Couteaux & Taxi 1952 Hellman 1952 Coers, 1953 Couteaux 1955) especially when long term of decalcification with EDTA is required Blood should be washed out because erythrocytes and serum are known to contain AChE and ChE, respectively (Alles & Hawes, 1940 \achmansohn & Rothenberg 1946) Therefore perfusion fixation is strongly recommended

Both AChE and ChE are demonstrated by Koelle s method \on specific

GACEK, R. R., NOMURA, Y. and BALOGH, K., JR., 1965. Acetylcholinesterase activity in the efferent fibers of the stato-acoustic nerve. *Acta Otolaryng* (Stockh.) 59, 541.

GOMORI, G., 1952. *Microscopic histochemistry. Principles and Practice.* p. 211. Univ. Chicago Press.

HELLWEG, K., 1952. The cholinesterase of the motor end-plate region. *J. Cell. Comp. Physiol.* 40, 421.

HILDING, D. and WERSÄLL, J. 1962. Cholinesterase and its relation to the nerve endings in the inner ear. *Acta Otolaryng.* (Stockh.) 55, 205.

IRELAND, P. E. and FARKASHIDY, J. 1961. Studies on the efferent innervation of the vestibular end organs. *Ann. Otol.* 70, 490.

ISHII, T., MURAKAMI, Y. and BALOGH, K., JR., 1967. Acetylcholinesterase activity in the efferent nerve fibers of the human inner ear. *Ann. Otol.* 76, 69.

KOELLE, G. B., 1954. The histochemical localization of cholinesterases in the central nervous system of the rat. *J. Comp. Neurol.* 100, 211.

— 1955. The histochemical identification of acetylcholinesterase in cholinergic, adrenergic and sensory neurons. *J. Pharm. Exp. Ther.* 114, 167.

NACHMANSOHN, D. and ROTHENBERG, M. A., 1945. Studies on cholinesterase. I. On the specificity of the enzyme in nerve tissue. *J. Biol. Chem.* 158, 653.

NOMURA, Y., GACEK, R. R. and BALOGH, K., JR., 1965. Efferent innervation of vestibular labyrinth. *Arch. Otolaryng.* (Chic.) 81, 335.

NOMURA, Y. and SCHUKNECHT, H. F. 1965. The efferent fibers in the cochlea. *Ann. Otol.*, 74, 289.

PEPLER, W. J. and PEARSE, A. G. E., 1957. The histochemistry of the esterases of the hypothalamic nuclei. *J. Neurochem.* 1, 193.

RASMUSSEN, G. L., 1946. The olivary peduncle and other fiber projections of the superior olivary complex. *J. Comp. Neurol.* 84, 141.

RASMUSSEN, G. L. and GACEK, R. R., 1958. Concerning the question of an efferent fiber component of the vestibular nerve of the cat. *Anat. Rec.* 130, 361.

RAVIN, H. A., TSOU, K.-C. and SELIGMAN, A. M. 1951. Colorimetric estimation and histochemical demonstration of serum cholinesterase. *J. Biol. Chem.* 191, 843.

ROSSI, G. 1961. L'acetylcholinesterase au cours du développement de l'oreille interne du cobaye. *Acta Otolaryng.* (Stockh.) Suppl. 170.

ROSSI, G., and CORTESINA, G., 1963. Research on the efferent innervation of the inner ear. *J. Laryng.* 77, 202.

— 1965. The efferent cochlear and vestibular system in Lepus cuniculus L. *Acta Anat.* (Basel) 60, 362.

— 1966. Acetylcholinesterase activity in the efferent fibres after destruction of the organ of Corti and afferent fibres. *Acta Otolaryng.* (Stockh.) 61, 488.

SCHUKNECHT, H. F., CHURCHILL, J. A. and DORAN, R. 1959. The localization of acetylcholinesterase in the cochlea. *Arch. Otolaryng.* (Chic.) 69, 549.

SMITH, C. A. and RASMUSSEN, G. L., 1963. Recent observation on the olivo-cochlear bundle. *Ann. Otol.* 72, 489.

SPOENDLIN, H. H. and GACEK, R. R., 1963. Electronmicroscopic study of the efferent and afferent innervation of the organ of Corti in the cat. *Ann. Otol.* 72, 660.

WERSÄLL, J., HILDING, D. and LUNDQUIST, P.-G. 1961. Ultrastruktur und Innervation der cochlearen Haarzellen. *Arch. Ohr.- Nas.- u. Kehlk.-Heilk.* 178, 106.

T. Ishii, M.D., Dept. of Otolaryngology
Faculty of Medicine, University of Tokyo
Hongo 7-chome, Bunkyo-ku,
Tokyo, Japan

Received December 20, 1966

ACKNOWLEDGMENT

The authors express thanks to Dr H F Schuknecht Dr K Balogh, Jr and Mr R S Kimura for their assistance with various aspects of this work.

ZUSAMMENFASSUNG

Es wurde die Perfusionsfixierung am „squirrel monkey" (Salmiri sciurea) durchgeführt. Seine Schläfenbeine werden in einer kalten Flüssigkeit von EDTA (Aethylendiamintetraacetat) dekalzifiert. Die Aktivität der Cholinesterase (ChE) wurde an den im Kryostat aufgearbeiteten Schläfenbeinschnitten gezeigt Durch spezifische Fermenthemmer konnte die Acetylcholinesterase (AChE) in den ef ferenten Bahnen des Innenohres identifiziert werden Die AChE konnte auch in den feinen verschiedengelegenen Fasern des N cochlearis nachgewiesen wer den die direkt über die Junktur zwischen Glia und Schwannscher Scheide verlaufen und die kein Teil des Rasmussenschen Bündels bilden Durch das Durchschneiden des N cochlearis ist festzustellen dass die Aktivität der AChE an den obengenannten feinen Nervenfasern verliert Es ist daher anzunehmen dass sie von efferenter Natur sind In dem Tunnelraum des Cortischen Organs konnte die AChE Aktivität in beiden Teilen also den Medialfasern und Basilar fasern nachgewiesen werden Diese beiden Bündel enthalten offenbar die ef ferenten Fasern

REFERENCES

ADAMS, C. W. M 1965 Histochemistry of the cell in the nervous system. In Neurohistochemistry (ed. C. W. M Adams) p 292 Elsevier Publishing Co Amsterdam, London and New York.

ALDRIDGE, W. N 1953 The differentiation of true and pseudo cholinesterase by organo-phosphorus compounds Biochem J 53 62.

ALLES, G A and HAWES, R C., 1940 Cholinesterases in the blood of man J Biol Chem 133 375

AUSTIN, L and BERRY W K., 1953: Two selective inhibitors of cholinesterase Biochem. J 53 ix

BALOGH, K J and NOMURA, Y 1964: A technique for the demonstration of acetylcholinesterase activity the inner ear after decalcification with EDTA J Histochem Cytochem 12 931

CHURCHILL, J A, SCHUKNECHT H F and DORAN R 1956 Acetylcholinesterase activity in the cochlea Laryngoscope 66 1

COËRS, C 1953 La détection histochimique de la cholinesterase au niveau de la jonction neuro-musculaire (I) Rev Belg Path 22 306

COUTEAUX, R 1955 Localization of cholinesterase at neuromuscular junctions. Int Rev Cytol 4 335

COUTEAUX, R and TAXI, J 1952: Recherches histochimiques sur la distribution des activités cholinestérasiques au niveau de la synapse myoneurale Arch Anat Micr Morph Exp 41 352

DEL BO M and CORTI A 1961 Distribuzione dell acetilcolinesterasi nell organo spirale della coclea di cavia Arch Ital Otol 72 44

DOHLMAN, G FARKASHIDY J and SALONEN, F 1958: Centrifugal nerve-fibers to the sensory epithelium of the vestibular labyrinth. J Laryng 72 984

Acta oto-laryngologica 64, 181-191 (1967)

UNILATERAL CALORIC HABITUATION OF NYSTAGMUS IN THE CAT

Effects on Rotational and Bilateral Caloric Responses

RUTH ANN MERTENS and W. E. COLLINS
Oklahoma City, Okla., U.S.A.

From the Civil Aeromedical Institute, Office of Aviation Medicine, FAA, Oklahoma City

Transfer of habituation of ocular nystagmus from unilateral calorization and from unidirectional rotation to bilateral calorization and to bidirectional rotation was investigated in 60 cats. Reductions in bilateral caloric responses were obtained independent of the intensity of the unilateral caloric habituating stimuli. Habituation to rotatory stimulation was *found to have no effect on responses to* bilateral caloric irrigations. Caloric habituation appeared to exert relatively little influence on rotation responses. Additional observations were concerned with retention of habituation, possible sex differences in nystagmic output, double irrigations and the influence of several post-tests on nystagmic responses in the unhabituated direction.

Several recent investigations have been concerned with questions of transfer-effects in habituation of ocular nystagmus. One such effect is that of stimulus transfer, i.e. transfer of habituation from one type of stimulation (e.g., rotatory) to another (e.g., caloric).

Maxwell, Burke & Reston (1922) and Hood & Pfaltz (1954) reported no decline in nystagmic responses to caloric stimuli after rotatory habituation. More recently, Collins (1964 *a* and *b*) investigated transfer from rotatory to caloric and from caloric to rotatory situations and obtained minimal transfer. Dunlap (1925), however, found a marked reduction in the response of rabbits to rotatory stimulation following a series of caloric irrigations with ice water. The absence of stimulus transfer effects (Collins, 1964 *a* and *b*) has been attributed to (1) differences in intensity of response produced by caloric irrigation and by angular acceleration, (2) differences in the pattern of neural excitation to unilateral and to bilateral stimulation. A study by Capps & Collins (1965) isolated certain of these differences and showed that, irrespective of the stimulus intensities used, a significant reduction in nystagmic responses *of the cat to unilateral* stimulation occurred after habituation to bilateral caloric irrigations. The

CALCIOPEN®-K

tabletter à 600 000 IE

Jamförande antibakteriella aktivitetsbestamningar har bekräftat de kliniska iakttagelserna att V penicillin fortfarande forsvarar sin plats som forstahandsprepa rat vid penicillinbehandling For att sakerstalla terapi svar även vid infektioner fororsakade av stammar med något mindre kanslighet for penicillin har man mer och mer overgått till hogre dosering

CALCIOPEN® K tabletten innehåller 600 000 IE Den är spolformad filmdragerad och lätt att svälja

DEKLARATION

Varje tablett innehåller
Phenoximethylpenicillinkalium 0 4 g
(respond 600 000 IE)

INDIKATIONER

Alla infektionssjukdomar förorsakade av penicillinkänsliga bakterier

KONTRAINDIKATION

Penicillinöverkänslighet

DOSERING

Vuxna 4 tabletter per dag (1 på morgonen 1 vid middagstiden och 2 till natten)

FÖRPACKNINGAR

Glas à 20 30 40 100 och 250 tabletter

Sjukhusforpackningar à 10×100 tabletter och 5 × 250 tabletter

LEO • HÄLSINGBORG

TABLE 1 *Schedule of test stimuli for each group*

R, right ear; *L*, left ear; *IW*, ice water irrigation; all temperatures are in °Centigrade.

Pre-tests	
Pre-1 Rotation (5 °/sec² for 12 sec accel. and decel.)	Pre-2: Bilateral caloric (*R*30° *L*46°C for 20 sec)
Habituation series (15 trials)	
Control groups	Experimental groups
Caloric: Bilateral caloric (*R*30° *L*46°C for 20 sec)	I Unilateral caloric (*IW* right for 15 sec)
Rotation: Rotation (5 °/sec² for 12 sec accel.; subthreshold decel.)	II Unilateral caloric (*R*44°C for 30 sec)
Combined: No habituation trials	III Unilateral caloric (*R*30 C for 30 sec)
Post-tests	
Post 1 Bilateral caloric (*R*30 *L*46°C for 20 sec)	Post 2: Rotation (5 °/sec² for 12 sec accel. and decel.)

quency) and duration of response (in seconds). Slowphase displacement values in millimeters were transformed into degrees of eye movement by means of the calibration constants (Capps & Collins, 1965).

PROCEDURE

Six groups of animals were used. A different series of 15 habituation trials was administered to each of five of these groups; the sixth group received no habituation trials (see Table 1). All animals were given identical pre- and post-tests (bilateral caloric irrigations and rotation trials). All trials, including the irrigation periods, were in total darkness.

Pre- and Post Tests

For all animals, the pre- and post-test rotation trials consisted of an acceleration (5 °/sec² for 12 sec), 2 min of constant velocity and a subsequent deceleration (5 °/sec² for 12 sec). Half of the subjects in each group received clockwise rotations and the remaining half received counter-clockwise rotations. Pre- and post test bilateral caloric stimulation consisted of simultaneous irrigations with water at a temperature of 30 C to the right ear and of 46 C to the left ear. Duration of stimulation was 20 sec.

Habituation Trials

Experimental groups

The habituation series for the three experimental groups consisted of 15 unilateral irrigations.

present study was *designed* to examine further some questions regarding transfer of habituation while attempting to evaluate the influences of intensity type of stimulus, *and unilateral vs bilateral stimulation*

METHODS

Subjects and restraint

Subjects were 60 mature cats, unused in previous experiments, and assigned randomly to six equal groups Restraint was accomplished by the method of Henriksson Fernández & Kohut (1961) with an adjustable restraining device For rotation trials the head was positioned so that a *line from the outer canthus of the eye to the tragus of the ear was in the* horizontal plane placing the lateral canals in the plane of rotation for caloric irrigations, *the head was inclined 45 upward from the horizontal*

Calori ation

Water baths equipped with Bronwill constant temperature circulators were used for 26 C, 30 C, and 46 C stimuli. For ice water irrigations temperature was 4 C Rubber tubing extended from the water baths to irrigation nozzles which were inserted into the animal s ears Rate of water flow was 15 cc/sec. Irrigation periods were controlled automatically by means of solenoids connected to a Hunter Interval Timer Model 111-C.

Rotation

The turntable (Collins & Huffman, 1964) was situated in a light-proof room A set of tiers allowed as many as three cats to be rotated simultaneously with their heads at the center of rotation (Collins & Updegraff 1966)

Recording

Needle electrodes inserted at the outer canthi of the cats eyes *picked* up corneo-retinal potentials, which were relayed through a terminal elec*trode board to the recorder* Two *Offner recorders* were used a Type TC for caloric trials and a Type R for rotation trials A 3 sec time constant was employed in amplification

Calibration

Prior to vestibular testing eye movement calibrations were *obtained by* means of an opto-kinetic stimulator (a steel drum with a striped interior) Eye *movements (opto-kinetic nystagmus)* induced by rotation of the drum at 24 /sec were recorded measured and used to calculate calibration constants

Scoring

For all trials three types of measures were obtained amount of *slow* phase eye displacement (in millimeters) number of nystagmic beats (fre-

TABLE 1 *Schedule of test stimuli for each group*

R, right ear *L*, left ear *IW* ice water irrigation All temperatures are in °Centigrade

Pre-tests	
Pre-1 Rotation (5°/sec² for 12 sec accel. and decel.)	Pre 2: Bilateral caloric (*R*30 *L*46°C for 20 sec)
Habituation series (15 trials)	
Control groups	Experimental groups
Caloric Bilateral caloric (*R*30 *L*46°C for 20 sec)	I. Unilateral caloric (*IW* right for 15 sec)
Rotation Rotation (5°/sec² for 12 sec accel., subthreshold decel.)	II. Unilateral caloric (*R*26°C for 30 sec)
Combined No habituation trials	III. Unilateral caloric (*R*30°C for 30 sec)
Post-tests	
Post 1 Bilateral caloric (*R*30 *L*46°C for 20 sec)	Post 2: Rotation (5°/sec² for 12 sec accel. and decel.)

quency) and duration of response (in seconds) Slowphase displacement values in millimeters were transformed into degrees of eye movement by means of the calibration constants (Capps & Collins, 1965)

PROCEDURE

Six groups of animals were used. A different series of 15 habituation trials was administered to each of five of these groups the sixth group received no habituation trials (see Table 1) All animals were given identical pre and post-tests (bilateral caloric irrigations and rotation trials) All trials, including the irrigation periods, were in total darkness.

Pre and Post Tests

For all animals, the pre- and post-test rotation trials consisted of an acceleration (5°/sec² for 12 sec) 2 min of constant velocity and a subsequent deceleration (5°/sec² for 12 sec) half of the subjects in each group received clockwise rotations and the remaining half received counter-clockwise rotations. Pre and post test bilateral caloric stimulation consisted of simultaneous irrigations with water at a temperature of 30 C to the right ear and of 46 C to the left ear Duration of stimulation was 20 sec.

Habituation Trials

Experimental groups

The habituation series for the three experimental groups consisted of 15 unilateral irrigations

present study was designed to examine further some questions regarding transfer of habituation while attempting to evaluate the influences of intensity type of stimulus and unilateral vs bilateral stimulation.

METHODS

Subjects and restraint

Subjects were 60 mature cats unused in previous experiments and assigned randomly to six equal groups Restraint was accomplished by the method of Henriksson Fernández & Kohut (1961) with an adjustable restraining device For rotation trials, the head was positioned so that a line from the outer canthus of the eye to the tragus of the ear was in the *horizontal* plane, placing the lateral canals in the plane of rotation for caloric irrigations, the head was inclined 45 upward from the horizontal.

Calorization

Water baths equipped with Bronwill constant temperature circulators were used for 26 C, 30 C, and 40 C stimuli For ice water irrigations temperature was 4 C. Rubber tubing extended from the water baths to irrigation nozzles which were inserted into the animal s ears. Rate of water flow was 15 cc/sec. Irrigation periods were controlled automatically by means of solenoids connected to a Hunter Interval Timer Model 111-C.

Rotation

The turntable (Collins & Hoffman 1964) was situated in a light proof room A set of tiers allowed as many as three cats to be rotated simultaneously with their heads at the center of rotation (Collins & Updegraff 1966)

Recording

Needle electrodes inserted at the outer canthi of the cats eyes picked up corneo-retinal potentials, which were relayed through a terminal electrode board to the recorder Two Offner recorders were used a Type TC for caloric trials and a Type R for rotation trials A 3-sec time constant was employed in amplification

Calibration

Prior to vestibular testing eye movement calibrations were obtained by means of an opto-kinetic stimulator (a steel drum with a striped interior) Eye movements (opto-kinetic nystagmus) induced by rotation of the drum at 24 /sec were recorded measured and used to calculate calibration constants

Scoring

For all trials three types of measures were obtained amount of slow phase eye displacement (in millimeters) number of nystagmic beats (fre-

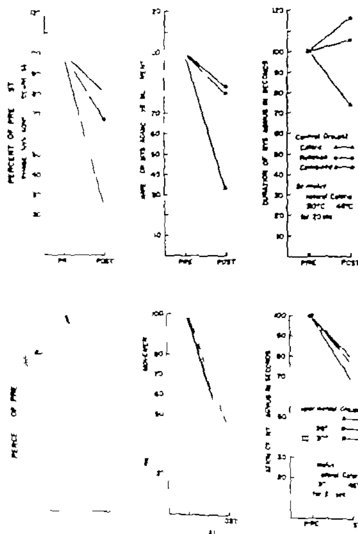


FIG. 1. Mean pre- and post-test bilateral caloric scores for slow-phase displacement, beat-frequency and duration of response for the experimental groups and for the control groups plotted as percentages of the pre-test.

expected variation in response. Ice water evoked the greatest amount of nystagmus, the 30° C irrigations produced the least, while the 26° C stimulus yielded an intermediate level of response.

Control groups

Both the Caloric and the Rotation Controls exhibited declines from Trial 1 to Trial 15 of the habituation series. (The Combined Control Group received no habituation trials.) The Caloric Control response decline was

Experimental Group I received an habituation series of ice water irrigations (4 C) applied to the right ear for 15 sec. The habituation trials for this group were of a greater intensity (resulted in greater response output) relative to the pre-trials than those of the other two experimental groups.

Experimental Group II received habituation trials consisting of irrigations with 26 C water administered to the right ear for 30 sec. These trials were of an intensity intermediate to those received by Groups I and III

Experimental Group III received an habituation series of irrigations with 30 C water applied to the right ear for 30 sec. This habituation series was the least intense of those received by the experimental groups

Control groups

Three control groups were used to provide a measure of the expected level of response after 15 bilateral calorizations (Caloric Control) 15 rotation trials (Rotation Control) and a period of no stimulation (Combined Control)

The Caloric Control Group received an habituation series of bilateral irrigations (30 C water to the right ear and 46 C to the left ear simultaneously for 20 sec) These 15 habituation trials were identical to the pre and post test bilateral irrigations received by all groups.

The Rotation Control Group received habituation trials which consisted of counterclockwise accelerations (5 /sec² for 12 sec) 2 min of constant velocity and subthreshold decelerations (0 15 /sec²) Thus, only eye movements in the same direction as those evoked by the caloric habituation trials were elicited

The Combined Control Group received no habituation trials. The animals remained in restraint for a period of time equal to that of an habituation series

The test schedule appears in Table 1 In all cases a time interval of 20 minutes between the start of successive trials was provided Animals were run in groups of four for the caloric trials and in groups of three for rotation trials whenever practicable All animals were in room illumination for at least five minutes prior to each trial

RESULTS

Habituation Trials

Experimental groups

All experimental groups evidenced response declines from Trial 1 to Trial 15 of the habituation series These reductions ranged from 76–83% for slowphase displacement, from 58–74% for beat frequency and from 15–38% for duration of response Response reduction was most marked during the early trials of the habituation series.

The three stimulus levels (ice water 26 C, and 30 C) produced the

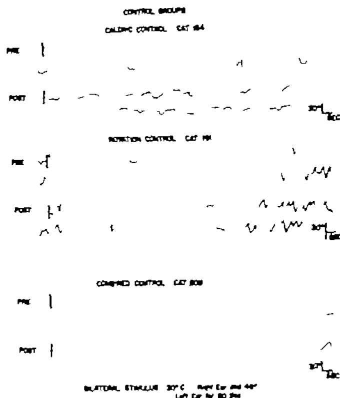


Fig. 3. Pre- and post-test tracings of bilateral caloric nystagmus for cats in the three control groups. Vertical bars mark the termination of the stimulus period.

trials, the groups also evidenced significant differences in pre- to post-test response reduction on all measures (.05 to .01 levels). (3) No significant differences in pre- to post-test response declines were found for responses to rotation in the direction opposite to those which occurred during the habituation series.

To ascertain which groups differed significantly in pre- to post-test response decline, t tests were executed on group pre- to post-test difference scores (% decline).

Bilateral calorizations

For bilateral caloric data, declines of the various groups were evaluated by comparing them statistically with the declines shown after a bilateral caloric habituation series (the Caloric Control Group) and with the reduction evidenced after a period of no stimulation (the Combined Control Group). For slowphase displacement, frequency and duration of response resulting from bilateral caloric stimulation, the response declines of the three experimental groups were as great as those of the Caloric Control Group. The three experimental groups and the Caloric Control Group also

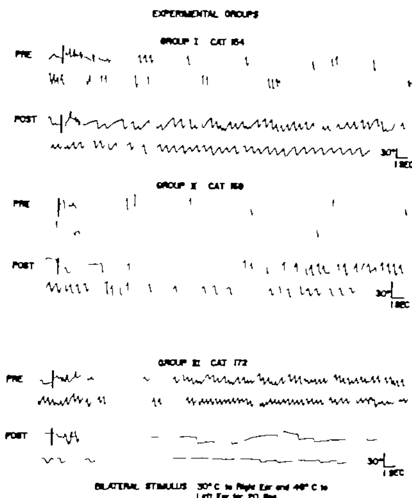


FIG. 2. Pre- and post-test tra i gs of bilateral caloric nystagmus for cats in the three xperim ntal groups V rtical bars mark the t rminati n f the timulus period

62% for slowphase displacement 50% for number of beats, and 2% for duration of response Corresponding reductions for the Rotation Control Group were 45% 23% and 15% respectively

Pre and Post Test Trials

Group differences

For the six groups, analyses of covariance were conducted on pre- and post test scores for (1) the bilateral caloric tests, (2) the rotatory stimuli eliciting responses in the direction stimulated during the habituation series, and (3) the rotatory stimuli eliciting responses in the direction opposite to that stimulated during the habituation trials. Separate analyses were executed for the three measures of nystagmus (slowphase frequency and duration) (1) Groups differed in pre- to post test response decline for bilateral calorizations on all measures (001 levels) (2) For responses to rotation in the same direction as those elicited during the habituation

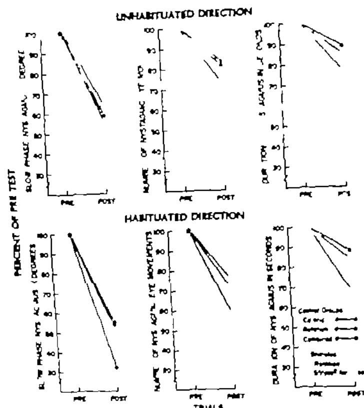


Fig. 5. Mean pre and post-test rotation scores for slowphase displacement, beat frequency and duration of response for the control groups plotted as percentages of the pre-test.

habituation stimulation) Therefore, it would seem that rotatory habituation did not transfer to bilateral caloric stimulation although both stimuli were of a bilateral nature

Fig 1 illustrates the group reductions in responses to the bilateral caloric irrigations for the experimental and control groups. Tracings of nystagmus during pre- and post test bilateral caloric responses are presented in Fig. 2 for the experimental groups and in Fig. 3 for the control groups.

Angular accelerations Response in the direction elicited during habituation

For rotation induced responses in the same direction as those elicited during the habituation series, pre- to post test changes shown by the groups were evaluated by comparing them with the declines found after a rotatory habituation series (the Rotation Control Group) and with the changes shown by the Combined Control Group Th Rotation Control Group declined significantly more in slowphase output than did all other groups

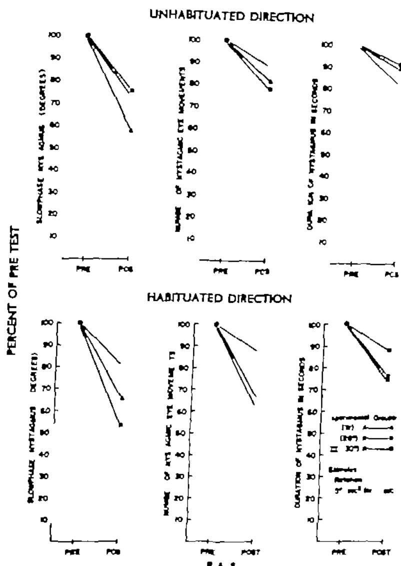


FIG 4 M an pre- and post test rotati n sc res for lowphase di placeme t beat fre qu ncy nd d rat on of response fo the expe iment l gro ps plotted as percentages of the pre-test

had significantly greater reductions in output on all measures than did both the Combined and the Rotation Control Groups (05 to 001 levels) while the latter two groups did not differ from each other on any measure Thus unilateral habituation stimuli produced as great a bilateral-caloric response reduction as did a series of bilateral caloric irrigations that is, unilateral caloric habituation transferred to bilateral caloric stimulation

The response reductions among experimental groups were not found to differ significantly on any measure Thus, intensity of the habituating stimulus did not appear to be a significant variable in this transfer of habituation

The Rotation Control Group showed significantly less pre- to post-test caloric response decline than did the three experimental groups and the Caloric Control Group (05 to 001 levels) while exhibiting a reduction no greater than that of the Combined Control Group (the group receiving no

reductions in pre- to post test slowphase displacement responses to rotation in the habituated direction than did the Rotation Control Group For slowphase displacement, the Rotation Control Group also showed a greater response reduction than did the Combined Control Group This was not the case for reductions in beat frequency and duration of response, however The unexpectedly small mean decline in beat frequency evidenced by the Rotation Control Group (significantly greater than the decline of only one other group) was due as noted earlier to depression of the mean by an atypical pre- to post-test increase, for this measure only shown by one animal in the group (this same animal showed a 25% pre- to post-test reduction in slowphase displacement)

The values plotted in Figs. 4 and 5 indicate that all groups which received caloric habituation trials (the three experimental groups and the Caloric Control Group) exhibited somewhat greater reductions for frequency and duration scores during post test rotations which elicited nystagmus in the same direction as that elicited during the caloric habituation trials (as opposed to rotation induced nystagmus in the direction opposite to that stimulated during the habituation series) This suggests that the caloric habituation series may have exerted some influence on subsequent responses to angular acceleration On the basis of these data, then, it is suggested that while caloric stimulation may have some influence on responses to rotation, a series of caloric irrigations apparently does not produce nearly so great a response reduction as does an equal number f rotation trials, even though the caloric stimuli are of much greater intensity (produce a greater response output) than the angular stimuli That this is the case whether the caloric stimuli employed activate only one labyrinth (the three experimental groups) or both labyrinths (the Caloric Control Group) indicates that differences in caloric and rotatory modes of activation may be at least partially responsible for failure to obtain t ansfer Another possible explanation for lack of intermodal tr nsfer lies in the difference between position of the otoliths during rotatory and during caloric timulation (heads were fixed in a position appro imately that f normal carriage for rotation trials and were elevated 45 for caloric irrigation) To the extent that the otoliths might influence semi-circular canal responses, tran fer of nystagmic habituation might be specific to a given position of the otoliths Data presented below in the section deal ng with rotatory-to-caloric transfer of habituation appear to support either of the possible explanation offered above

Transfer of R tatory H bituation to Bilateral Caloric Stimulation

Results of the present investigation demonstrate a failure to obtain transfe of response declines from rotatory habituation to bilateral caloric stimulation. This is readily apparent in the values plotted for the Rotation Control Group in Fig. 1 That no significant rotatory-to-caloric transfer

(.05 to .001 levels). (The Combined Control Group also declined significantly more than Experimental Group III.) For beat frequency in the habituated direction, the response reduction of the Rotation Control Group was statistically greater than that of only Experimental Group III (.05 level). (Group III also declined significantly less than did Groups I and II.) The fact that the Rotation Control Group did not exhibit a greater mean decline in beat frequency was due to a large pre- to post-test increase in the number of eye movements from one animal, the post test response consisting of a large number of low-amplitude nystagmic beats. The decline in duration of response for the Rotation Control Group was not statistically greater than that for any of the other groups. (Experimental Group I showed a significantly greater reduction in duration of response than did Experimental Group III or the Combined Control Group.) Thus, frequency and duration data allow for no unequivocal conclusion concerning caloric-to-rotatory transfer, as the decline of the Rotation Control Group on these measures was statistically no greater than that shown by the group receiving no habituation trials (the Combined Control Group). On the basis of the slowphase data obtained, however, it would appear that habituation to caloric stimulation has little effect on rotatory responses.

Figs. 4 and 5 demonstrate pre- to post-test reductions for rotation responses in both directions for the experimental and control groups, respectively.

DISCUSSION

Transfer of Unilateral Caloric Habituation to Bilateral Caloric Stimulation

The data indicate that unilateral caloric habituation transfers to bilateral caloric stimulation. All three experimental groups (those groups receiving unilateral caloric habituation trials) exhibited as great a bilateral-caloric response reduction as did the Caloric Control Group (the group receiving bilateral caloric habituation trials).

While the three intensities of unilateral stimuli resulted in three different levels of response-output on early trials, the intensity factor did not appear to influence transfer of unilateral habituation to bilateral stimulation within the intensity limits described here, as the three experimental groups did not differ in amount of post test bilateral-caloric response reduction. This finding parallels that of Capps & Collins (1965) who indicated that intensity of the habituating stimulus was a negligible factor in their examination of bilateral-to-unilateral transfer of caloric habituation.

Transfer of Caloric Habituation to Rotatory Stimulation

All groups receiving caloric habituation trials (the three experimental groups and the Caloric Control Group) evidenced significantly smaller

reductions in pre- to post-test slowphase displacement responses to rotation in the habituated direction than did the Rotation Control Group For slowphase displacement the Rotation Control Group also showed a greater response reduction than did the Combined Control Group This was not the case for reductions in beat frequency and duration of response however The unexpectedly small mean decline in beat frequency evidenced by the Rotation Control Group (significantly greater than the decline of only one other group) was due as noted earlier to depression of the mean by an atypical pre- to post-test increase for thi measure only shown by one animal in the group (this same animal showed a 23% pre- to post-test reduction in slowphase displacement)

The alues plotted in Figs. 4 and 5 indicate that all groups which received caloric habituation trial (the three experimental groups and the Caloric Control Group) exhibited somewhat greater reductions for frequency and duration scores during post-test rotations which elicited nystagmus in the same direction as that elicited during the caloric habituation trials (as opposed to rotation-induced nystagmus in the direction opposite to that stimulated during the habituation series) This suggests that the caloric habituation series may have exerted some influence on subsequent responses to angular acceleration On the basis of these data, then, it is suggested that while caloric stimulation may have some influence on responses to rotation, a series of caloric irrigations apparently does not produce nearly so great a response reduction as does an equal number f rotation trials, even though the caloric stimuli are of much greater intensity (produce a greater response output) than the angular stimuli. That this is the case whether the caloric stimuli employed activate only one labyrinth (the three experimental groups) or both labyrinths (the Caloric Control Group) indicates that differences in caloric and rotatory modes of activation may be at least partially responsible for failure to obtain transfer Another possible explanation for lack of intermodal transfer lies in the difference between position of the otoliths during rotatory and during caloric stimulation (heads were fixed in a position approximately that of normal carriage for rotation trials and were elevated 45 for caloric irrigation) T the extent that the otoliths might influence semi-circular canal responses, transfer of nystagmic habituation might be specific to a given position of the otoliths. Data presented below in the section dealing with rotatory-to-caloric transfer of habituation appear to support either of the possible explanations offered above

Transfer of Rotatory H bituation to Bilateral Caloric Stimulation

Results of the present investigation demonstrate a failure to obtain transfer f response declines from rotatory habituation to bilateral caloric stimulation This is readily apparent in the values plotted for the Rotation Control Group in Fig. 1 That no significant rotatory-to-caloric transfer

was obtained is in agreement with findings of other investigators (Collins, 1964 b Hood & Pfaltz, 1954 Maxwell Burke & Reston 1922)

The failure to obtain transfer however cannot be ascribed to differential stimulation of one or both labyrinths by the rotatory and caloric stimuli, as the caloric irrigations used in the transfer tests were bilateral. Also, data already reported (Capps & Collins, 1965) as well as findings of this study indicate that intensity of the habituating stimulus appears to be a negligible factor in transfer of habituation within the intensity limits employed Specifically in the present investigation caloric irrigations (both unilateral and bilateral) although of a greater intensity than the angular stimuli used appeared to have only a slight effect on responses to rotation In addition intensity of the habituating stimuli was found not to influence unilateral to-bilateral caloric transfer of habituation The present lack of transfer therefore cannot be explained adequately as being a result of the fact that the rotation trials (habituation series) were relatively mild stimuli in comparison to the pre- and post-test bilateral caloric irrigations. It would seem then that failure to obtain rotatory to-caloric transfer may depend more upon differences in the rotatory and caloric modes of response-activation or upon some otolithic influence than upon relative intensities or differential stimulation of the labyrinths.

Additional Observations

Sex differences

Capps & Collins (1965) reported statistically significant (05 level) sex differences (favoring female cats) in amount of slowphase output and frequency of nystagmus to unilateral irrigations The 22 male cats in this study were compared by t test with a group of 22 female cats randomly selected from the remainder of the group Although females were found to have greater average pre-test response output to bilateral caloric irrigation than did males (2978 vs 1842 for slowphase displacement 175 vs 121 for frequency) these differences fell just short of statistical significance at the 05 level ($t = 1\ 09$ and $1\ 87$ where $p_{05} = 2\ 02$) Female cats also showed slightly greater average responses to the pre test rotation (673 vs. 610 for slowphase displacement 33 vs 32 for frequency) but these differences were clearly not significant ($t = 0\ 71$ and $0\ 40$ where $p_{05} = 2\ 02$) The present findings thus indicate that if sex differences in vestibular functioning of the cat exist (*a*) they are probably limited to caloric stimulation and related more to physical factors influencing thermal conduction that to vestibular function *per se* and (*b*) there is considerable overlapping of the male and female response distributions

Double Irrigations

When water of the same temperature is applied to both ears simultaneously (double irrigations) the opposing stimuli theoretically cancel any

horizontal nystagmus (see Collins, 1965) Upon administering double ir rigations to a group of cats already habituated to unidirectional bilateral calorizations, Capps & Collins (1965) found no effect of the habituation series on responses. To examine further the possible influence of unidirec tional caloric habituation on responses to double irrigati ns, animals in Experimental Group II (20 C) and the Combined Control Group (no habituation stimulation) were administered two double irrigations immediately following the post 2 trial Half of the animals received stimulation with water of 30 C on the first trial and 46 C on the second trial The order was reversed for the remaining animals.

Some clearly-defined nystagmus was obtained on 6 of 20 records from the Combined Control Grouy and on 11 of 20 for Experimental Group II For the Combined Control Group 3 records evidenced nystagmus in the "unhabituated direction 1 in the habituated" direction and 2 showed "reversing" responses (responses first in the unhabituated direction and then in the habituated direction) For Experimental Group II 8 records yielded responses in the unhabituated direction 2 were in the habituated direction, and 1 case of "reversing" occurred. Seven of the eight responses in the habituated direction were accounted for by four cats Thus, although animal exposed to an habituation series produced more tracings with some clear nystagmus than did animals receiving no habituation stimulation data of the present study offer no unequivocal evidence that unilateral caloric habituation influences responses to double irrigations"

Additional post-trials

A single post-test for transfer may not always be sufficient to allow habituation transfer-effects to evidence themselves (Capps & Collins, 1965) In order to explore this possibility the Rotation Control Group was given a series of eight bilateral caloric irrigations after completion of the post 2 otation trial These consisted of f ur trials during which responses were elicited in the habituated direction (*R30 L46*) and four trials during which responses in the unhabituated direction (*R46 L30*) were evoked. The two stimuli were applied alternately from trial to trial Irrigation periods were 20 sec In addition, the Caloric Control Group was given a series of five rotation trials following (and identical to) the post 2 trial Half f the animals were rotated CW and half were rotated CCW

A can be seen in Fig. 6, responses in both the habituated and un habituated direction fall off to about th same level after four irrigations or after fi rotations (taking into consideration the pre- and post-test trial) This provides confirmation that rotatory habituation trials had little influence on responses to calori irrigation and that bilateral caloric habitu tion had little effect on rotation-induced responses. Transfer effects, therefore were not merely "masked" by such factors as novelty d to a change of the stimulus.

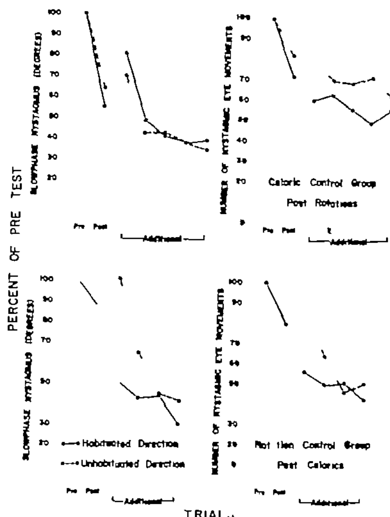


FIG. 6 Mean slowphase displacement and beat *frequency scores for the* additional post tests plotted as percentages of the pre-test. The upper figures illustrate values for post rotations, and the lower figures for post-calorics.

Retention trials

Reports indicate that habituation of nystagmus is retained for considerable periods of time (e g., Capps & Collins, 1965 Henriksson Fernández & Kohut 1961) In order to obtain some further information regarding retention *half of the animals in experimental groups I (ice water) and III* (30 C) were tested at one week following and the remaining half at one month following their habituation series. Eight bilateral caloric irrigations were administered at rates of 15 cc/sec for 20 sec Four irrigations produced responses in the direction previously habituated (*R*30 *L*46) and four yielded nystagmus in the opposite direction (*R*46 *L*30) The two stimuli were alternated from trial to trial

Data obtained after one week and after one month of rest are plotted in Fig 7 As data for Groups I and III were similar results were combined One week after the habituation series the first test in the habituated direction showed recovery to 60% of the original pre test level The one-month retention curves indicate *more recovery (75% of the pre-test) but*

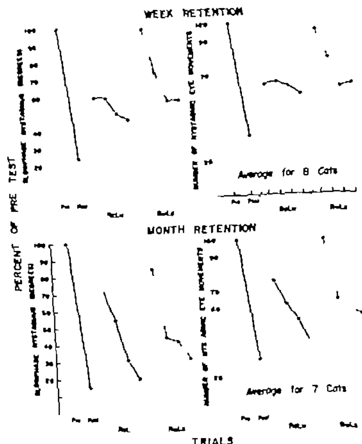


FIG. 7. Mean slow-phase displacement and beat frequency scores at one week and at one month following the habituation series plotted as percentages of the pre-test.

drop off more rapidly than the "one week" retention curves. Unhabituated direction slow-phase responses at one month also decline more rapidly than at one week, reaching 30% of pre-test after 4 trials. Initial differences between the response curves for the habituated and unhabituated directions indicate the directional specificity of habituation. However, responses in both directions declined steadily with repeated stimulation.

ACKNOWLEDGMENT

A major portion of the data presented here was submitted by the first-named author to the Psychology Department, University of Oklahoma, in partial fulfillment of the requirements for the degree of Master of Science. Grateful acknowledgment is made of the assistance rendered by Bill Updegraff, Sue Grounds, Nancy Rice, Jean Heaton, and David Schroeder.

RÉSUMÉ

La transmission de l adaptation nystagmique de *la calorisation unilatérale et* de la rotation à un côté à la calorisation bilatérale et à la rotation aux deux côtés fut examiné avec un groupe de 60 chats. Des réductions des réponses caloriques bilatérales furent obtenues sans égard pour l intensité des stimules caloriques unilatérales d adaptation L adaptation à la stimulation rotatoire n in fluença pas les réponses à des irrigations caloriques bilatérales. L adaptation calorique parut à exércer peu d influcnce sur les réponses à la rotation. Des observations additionnelles regardaient la retention de l adaptation, les dif ferences de réponse entre les sexes, les irrigations des deux oreilles et l influence de plusieurs épreuves sur les réponses du côté non habitué

ZUSAMMENFASSUNG

Das Auftreten der Nystagmusgewöhnung von unilateraler Kalorisierung und von angularer Beschleunigung in eine Richtung nach bilateraler Kalorisierung und nach angularer Beschleunigung in beide Richtungen wurde an einer Gruppe von 60 Katzen untersucht. Abschwächungen der bilateralen kalorischen Reaktio- nen wiesen sich unabhängig von der Stärke der unilateralen kalorischen Gewöh nungsreizungen auf Gewöhnung an angulare Beschleunigung wirkte nicht auf die durch bilaterale kalorische Ohrspülungen ausgelösten nystagmischen Reak tionen Es schien dass kalorische Gewöhnung nur kleinen Einfluss auf die Beschleunigungsreaktionen hatte Weitere Untersuchungen vermittelten Informa tionen in bezug auf Gewöhnungsbeibehaltung, mögliche Geschlechtsunterschiede im Ausmasse der Reaktionen beidseitige Ohrspülungen" und den Einfluss mehrerer Prüfungen auf die Nichtgewöhnungsrichtungsreaktionen

REFERENCES

CAPPS, M. J and COLLINS, W E., 1965 Effects of bilateral c l ri h bita tion on v tibular nystagmu in th cat *Acta Ot laryng* (Stockh.) *59* 511

COLLI s, W E., 1964 *a:* Ny tagmu responses of the cat t rotati and t directi nally equi alent nd n n-equival nt stimuli ft r u ilateral al ric habituati *Acta Oto- l ryng* (Stockh) *58* 247

— 1964 *b:* P imary secondary and al ic nystagmus of the c t f ll wi g habituati n to rot tion *J Comp Physiol Psych l* *57* 417

— 1965 Effect f doubl irrig tion n th l ric nystagmu f the cat *Acta Oto- laryng* (Stockh) *59* 45

COLLINS, W E., d HUFFMAN, W H 1964 Design nd perf rma ce ch racteristics of a mechanicall d i en estibul timulator *CARI R port* No. 64 15, Okl homa Cit Okl h ma.

COLLINS, W E., and UPDEGRAFF B P 1966 A compa ison of n t gm habituation in th cat nd th dog *Acta Otol yng* (Stockh) *6** 19

CR MPTON, G H 1962 Directi nal imbalance f estibul y t gmu i th cat f ll w ing repeated unidirectional gular ccelerati n. *A l Otolaryng* (Stockh.) *55* 41

DUNLAP L., 1925 Adaptation of n tagmu t repeated cal ric tim l ti l rabbits. *J Comp. Physiol Psychol* *5* 485

Henriksson, N. G., Fernández, C., and Kohut, R. I. 1961: The caloric test in the cat. Acta Otolaryng. (Stockh.) 53, 21.
Hood, J. D. and Pfaltz, C. R., 1954: Observation upon the effect of repeated stimulation upon rotational and caloric nystagmus. J. Physiol. 124, 130.
Maxwell, S. S., Burke, U. L., and Reston, C., 1922: The effect of repeated rotation on the duration of after-nystagmus in the rabbit. Amer. J. Physiol. 58, 432.

AC 118 CAMI-FAA
Oklahoma City Okl. 73125 U.S.A.

Received January 8, 1967

Acta oto-laryngologica 64 298–312 (1967)

NEOMYCIN INDUCED CHANGES IN THE ULTRASTRUCTURE OF THE OLFACTORY EPITHELIUM OF THE GUINEA PIG

A ARSTILA and J WERSÄLL
Turku and Stockholm

From the Department of Anatomy University of Turku Finland and the Department of Otolaryngology Karolinska Sjukhuset and King Gustaf V Research Institute Stockholm

The present work demonstrates the toxic action of neomycin on the olfactory epithelium after both local and intraperitoneal administration The most severe changes are observed in the proximal part of the epithelium of intraperitoneal administration whereas the changes are more extensive near the free surface of the epithelium after local administration In the supporting cells there is an increase in the number of mitochondria lysosomes, double membranes, myelin figures and agranular vesicles, and a lengthening of the microvilli In the receptor cells there is degeneration of the mitochondria and the outer membranes and vesicles of the cilia as well as an increased formation of centrioles. The significance of these changes for the olfactory function is discussed

INTRODUCTION

Antibiotics, produced by various streptomyces strains, have been shown to cause degeneration of the sensory cells of the inner ear when given in doses that result in high blood concentration of the antibiotics (Hawkins & Lurie, 1952 1953 Hawkins, 1959 Duvall & Wersall 1964 Lundquist & Wersall 1966 Kohonen, 1965)

The local effect of all these antibiotics has also been shown to be toxic to the inner ear cells (Schuknecht 1956) Although clinical experience has revealed that neomycin might cause olfactory disturbances when applied locally in the nose, no experimental studies have so far proved that such an effect occurs The present work was designed to demonstrate the local action of neomycin on olfactory epithelium and the reaction of this epithelium to the general administration of the drug

MATERIAL AND METHODS

Intraperitoneal administration

Six guinea pigs, weighing from 400–600 g, were treated with intraperitoneal administration of 400 mg of Mycifradin sulphate per kg body weight (UpJohn) for seven consecutive days.

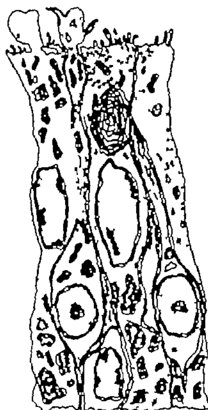


Fig. 1. Survey picture demonstrating changes in the basal part of the olfactory epithelium after intraperitoneal injection of neomycin in toxic dose.

Local administration

Fourteen guinea pigs, weighing from 400–600 g, were treated daily with Mycifradin sulphate for 21 consecutive days. 50 mg of Mycifradin sulphate in one milliliter of physiologic saline were injected three times daily into both nostrils of a guinea pig. The injection was made by using a narrow plastic tube connected to a syringe in order to get the solution as near the olfactory mucosa as possible. During or after the injection the guinea pigs were provoked to sneeze which secured the contact of the solution with the olfactory epithelium.

Controls

In both experiments control animals were treated in respectively the same way with physiologic saline solution. Altogether 10 animals were used.

After the planned dose was given the animals were killed by rapid decapitation and the olfactory mucosa was fixed with 1% phosphate-buf

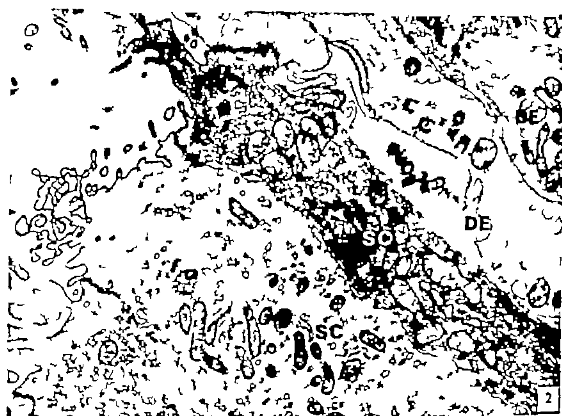


FIG. 2 Intraperitoneal admini trati : Chang of arvi g degree i the cytoplasm of th upporting cell (*SC*) In three dj cent dendrit s (*DE*) n apparent incre se in th number f centrioles is seen. × 12 000

fered osmium tetroxide (Millonig 1961) and embedded in Epon The sections were stained with uranyl acetate (Watson 1958) and lead citrate (Reynolds, 1963) and photographed with a Siemens Elmiskop I electron microscope

RESULTS

Intraperitoneal Administration

After the administration of neomycin for one week no apparent changes were observed in the distribution of different cell types or in the size of these cells However typical intracellular changes were observed in each of the cell types, and it appeared that most of these changes were located in the proximal portion of the epithelium whereas the changes near the free border of the epithelium were smaller (Figs. 1 and 2)

The supporting cells

The appearance of the nuclei of these cells was normal and the changes were restricted to the cytoplasm Usually there are a few round single-membrane-limited inclusion bodies in the basal feet of these cells (Fig 3) In this experiment, however abundant single-membrane-limited inclusion

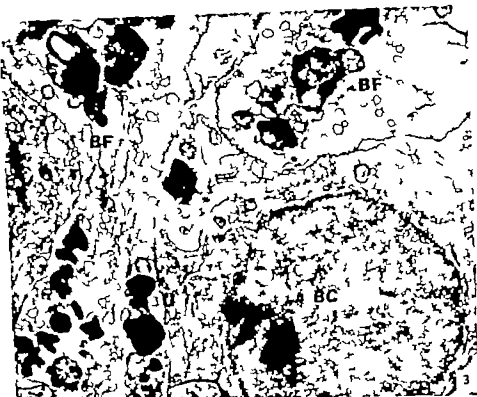


Fig. 3. Intraperit neal admi istratio Basal feet (*BF*) of the pporti g cell ha ing strongly osmiophilic bodies, some of which are limited by singl membrane On the right basal cell (*BC*) with ormal ppearance. 12,000

bodies (SMLIB) as well as highly osmiophilic myelin figures were seen, which were accumulated in the proximal part of the basal feet near the basement membrane. The inner structure of these bodies consisted of dark, osmiophilic myelin figures as well as degenerated cytoplasmic matrix, thu resembling th cytosegrosomes (Fig. 4) The myelin figures resembled the SMLIB, but in these figures the highly osmiophilic inner structure seemed to cover the outer single membrane Changes were also seen in the number of mitochondria in the basal feet Some of them had lost their inner cristae and in some others small dark osmiophilic bodies were observed instead of the cristae In the cell body the same kind of changes as those in the basal feet, but maller were noted. In the apical portion of these cells the hanges were however of a different type Th characteristic change here w the great increase in the number of tubules and vesicles compared with the n rm l structure of thi part of th cyt plasm These tubules and vesicles seemed to originate from a large whorl of doubl m mbranes (Fig 5) which w ften seen in th mitochondria The microvilli and large bulb-l ke extensions of the free border ppeared to be normal in thi experiment.



FIG. 4 Intraperitoneal administration: A larg ingle-membrane-limited inclusio body in a supporting cell containing o miophilic deb is. 18,000

The sensory cells

In these cells the greatest changes were seen in the cytoplasm around the nucleus Usually some small SMLIB are found in this part of the cytoplasm In this experiment their number was increased (Fig 6) Changes were also observed in the number of mitochondria though most of them had a normal appearance The changes in the mitochondria were of the same type as those in the mitochondria of the supporting cells, that is, the loss of inner cristae and the appearance of dark material instead of the cristae No changes were observed in the axons of these cells In some mitochondria of the dendrites the same kind of changes were seen as those in the mitochondria of the cell body Usually only some centrioli are seen even in the longitudinal sections of the dendrites and most of them are situated in the proximal portion of the terminal swelling In this experiment as many as 20 transverse sections of the centrioli could be observed in a number of the longitudinal sections of the dendrites (Figs 2 and 7) Although some of these transverse sections may belong to the same centrioli, there seemed to be an increase in their total number

No definite changes were seen in the terminal swellings or in the structure of the cilia



Fig. 5. Intraperitoneal administration. A whorl of double membranes in the cytoplasm of supporting cell. 21,000

The basal cells

The changes were small compared with those in the supporting cells and were restricted t an increase in the number of SMLIB around the nucleus.

Local Administration

The changes in this part of the experiment were greater compared with those connected with the intraperitoneal administration. This time the changes appeared to be centered near the free border of the epithelium, whereas in th intraperitoneal administration they were centered in the proximal part of the epithelium near the basement membrane (Figs. 8 and 9

The upporting cells

Great changes in these cell were observed also in this experiment but their severity varied from one cell t another and there was a complete series of changes, from such cells where the whole structure of the cell was changed, to those cells i which only small changes were observed.



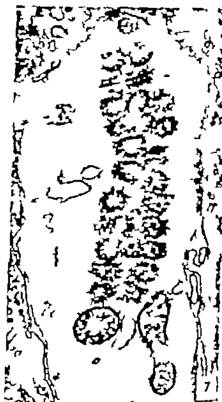


FIG 6 Intraperitoneal administrati n Osmiophilic bodies nd degen rated mitochondria in th cytoplasm of the receptor c lls. 12,000

FIG. 7 Intraperitoneal administration: A pronounced increase in the number of cen trioles in the dendrit of a receptor cell 24 000

No regularity however could be noted in the distribution of changes among the cells undergoing various stages of degeneration but the degeneration seemed rather to occur at random between individual cells Most changes were seen in the apical cytoplasm of these cells. The number of microvilli had increased and also their length from about 1 μ to 3–4 μ so that they appeared to form a dense thicket like structure under which the terminal swellings of the sensory cells were situated (Figs. 9 and 10) In the apical cytoplasm the changes were pronounced The number of mitochondria had increased many times so that the whole apical cytoplasm was, in many cases, crowded with them (Figs. 11 and 12)

The majority of these crowded mitochondria seemed to have a normal ultrastructure and only in those cells where the greatest overall changes occurred did a number of mitochondria appear to be degenerated in the same way as previously described in connection with intraperitoneal ad ministration Between the mitochondria a large number of tubules were seen which, in the apical portion of the cytoplasm and in the bulb-like ex tensions of the free border of the cell formed large whorls of membranes

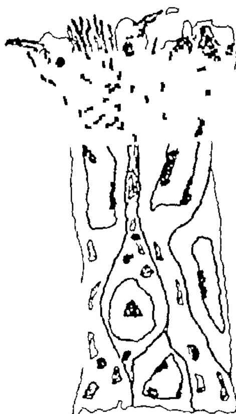


Fig. 8. Schematic drawing of olfactory epithelium demonstrating the distribution of the pathological changes in the neomycin-damaged epithelium.

which, in many cases, filled the whole apical cytoplasm. There was also an increase in the number of ribosomes in this part of the cytoplasm. In most cells no increase was observed in the number or structure of the SMLIB. However, there were also cells in which the same kind of changes were observed as those occurring after intraperitoneal administration of neomycin. In addition to these SMLIB there were also large double membrane-limited inclusion bodies, inside which the degenerated cytoplasmic matrix could be seen. Besides these changes in the cytoplasmic organelles the whole ground matrix of the cytoplasm appeared to be darker than normally. This opacity appeared to be due to the accumulation of electron-dense amorphic material in the ground substance between the organelles. This material was very conspicuous in the bulb-like extensions of the cytoplasm, which were devoid of cytoplasmic organelles.

In the proximal portion of these cells the changes were smaller and restricted to a slight increase in the number of SMLIB. No changes were observed in the nuclei of these cells.



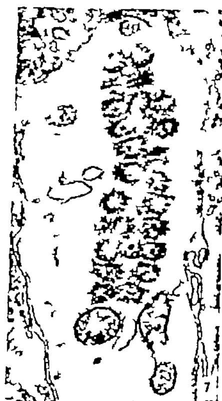


Fig. 6 Intraperitoneal admini tr ti n: Osmiophilic bodie and degen rated mitochondria in th cytoplasm of the recepto cells × 12,000

Fig. 7 Intraperitoneal administrati n A pron nced increa e i th number f centrioles in th dendrite of a recept cell 24 000

No regularity however could be noted in the distribution of changes among the cells undergoing various stages of degeneration, but the degeneration seemed rather to occur at random between individual cells. Most changes were seen in the apical cytoplasm of these cells. The number of microvilli had increased and also their length from about 1 μ to 3–4 μ so that they appeared to form a dense thicket like structure under which the terminal swellings of the sensory cells were situated (Figs. 9 and 10) In the apical cytoplasm the changes were pronounced The number of mitochondria had increased many times so that the whole apical cytoplasm was, in many cases, crowded with them (Figs. 11 and 12)

The majority of these crowded mitochondria seemed to have a normal ultrastructure and only in those cells where the greatest overall changes occurred did a number of mitochondria appear to be degenerated in the same way as previously described in connection with intraperitoneal ad ministration Between the mitochondria a large number of tubules were seen which, in the apical portion of the cytoplasm and in the bulb-like ex tensions of the free border of the cell formed large whorls of membranes



Fig. 10. A higher magnification of the free border of the former cells. In the cytoplasm of the supporting cell an increase in the number of ribosomes is seen. In the terminal swelling of the receptor cell both mitochondria and cilia membranes show less degeneration. ×27,000.

In the cell body the greatest changes were seen in the mitochondria. This time most of them were swollen, having empty spaces between the cristae. Osmiophilic bodies similar to those which appeared after intraperitoneal administration were only rarely observed. The possibility of these changes being due to fixation artefact did not seem probable in view of the well preserved mitochondria in nearby supporting cells. Furthermore the number of single-membrane-limited inclusion bodies was increased in the cell body. In the dendrites similar changes were seen in the mitochondria to those in the cell body. An increase in the number of small agranular vesicles of the dendrites was also observed.

In the terminal swellings the same kind of changes were seen as in the dendrites, that is, the degeneration of the mitochondria and an increase in the number of granular vesicles (Fig. 13). Occasionally lysosome-like bodies, which do not occur normally, were also observed in the terminal swellings. The degree of degeneration seemed to vary substantially between individual terminal swellings and in nearby swellings some could be badly degenerated whereas others had preserved a structure that was nearly normal. In the extensively degenerated terminal swellings the number of cilia



FIG. 9 Local dmi istrati n A urv y pict re of th h ges Th upport ng cell (SC) n th l ft ha dark ppearance d to th pron need ha ges n the cyto- pla m, where tl pporti g cell (SC) l th middl l bett r pres rved. I the cyt pla m of the recepto cell (RC) merou l gle-m mbra l mit d incl l bodies re l bl whi h esembl multi esicula bodies d lysos mes 6000

The sensory cells

More extensive changes were seen also in these cells than after intra peritoneal administration Most of the changes were situated in the cyto- plasm around the nucleus, in the dendrites, and in the terminal swellings, whereas the axons and the nuclei appeared to have a normal appearance



Fig. 10. A higher magnification f the free border f the former cells. In the cytopl m of the supporting cell an increase i the mber of ribosomes i seen I the terminal swelling of the recept cell both mitochondria nd cilia members s how les degen tion. 27,000

In th cell body the greatest changes were seen in the mitochondria. This time most of them were swollen, having empty spaces between the cristae Osmiophilic bodies similar t those which appeared after intraperitoneal admini tration were nly rarely observed The possibility of these changes being due to fixation artefact did not seem probable in view of the well preserved mitochondria in nearby supporting cells. Furthermore the num ber of single membrane-limited inclu ion bodies was increased in the cell body In the dendrites similar changes were seen in the mitochondria to those in the cell body An increase in the number of small agranular ves icles f the dendrites wa also observed

I the termi l swelling the same kind of changes were seen as in the dendrites, th t is, the degeneration of the mitochondria and an increase in the number of granular esicles (Fig 13) Occasionally lysosome-like bodies, which do not occur normally were also observed in the terminal swellings. The degree of degen ration seemed to vary substantially between ndi idual terminal wellings nd in nearby swellings some could be badly degenerated wherea others had preserved a structure that was nearly nor m l In the extensively degenerated terminal sw llings the number of cilia



FIG 11 Local dmi istrati i An pl al porti n of a upporti g cell with a great i crease in the numbe f mitochondria, and the occurrence of whorl f membranes between th m. Note th d k granular appearance of th cyt pla mic matrix 9000

seemed to have decreased and the whole swelling had lost its normal pine-like appearance which now seemed irregular Usually one or two microvilli can be seen in these swellings, but in this case long microvilli (Fig 12) were observed in the badly degenerated swellings, as if the cilia had been replaced by them In the cilia the changes were most apparent in their membranes (Figs. 10 and 12) which appeared to be irregular and winding in the longitudinal sections of the proximal and distal shafts In transverse sections these shafts had also lost their regular round structure and the membranes had spikes, which gave them an irregular contour In spite of the changes in the ciliary membranes the fibers of the proximal shaft had preserved their characteristic 9+2 pattern and no differences were noted in the appearance of the fibers compared with their normal structure The triplet structure of the basal bodies was also normal In addition to the degeneration of the membranes, extensive changes were seen in the tips of the cilia and in their lateral projections Usually the tips had swelled to large empty structures, in which either no smaller vesicles could be found



Fig. 12. Local administration. A accumulation of mitochondria in the apical part of the degenerated, dark, supporting cells (SC). Degeneration of the mitochondria in the central terminal swelling (TS) in which micro filaments (arrow) is observed. In the terminal swelling on the left longitudinal section of double cilium (DC). In the upper part of the picture except tips of the cilia (TC). 9000

or only one or two larger vesicles (Fig. 12). Similarly in the lateral projection only one or two irregular shaped vesicles were seen instead of the numerous round, smaller vesicles usually found. Despite these changes in numerous cilia there were also others in which no changes were seen. A characteristic feature in this part of experiment was the frequent occurrence of double cilia, in which the proximal shaft had two 9+2 fiber pattern (Fig. 12).

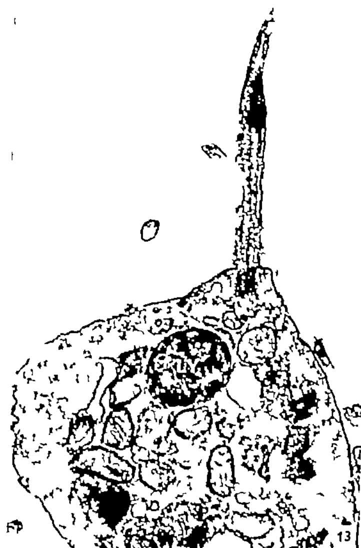


FIG. 13 Local adml l tration Pron unced changes in the t rminal sw lli g, sh wing numerous da k mitochond l vacuoles nd a lys s m (*LY*) The llia are rathe nor mal in ppearance Not th continuati n of the central fibers of the pro imal shaft up t the dist l h ft. 300 000

DISCUSSION

The present work demonstrates that neomycin can act as a toxic agent on the olfactory epithelium both when injected and when given locally When injected neomycin apparently reaches the epithelium through the capillary route as the effect is found to be most severe closest to the basement membrane of the epithelium The effect was much more rapid than when the drug was given locally This probably means that injection caused a higher concentration in the tissues. It is rather difficult to calculate the actual amount of neomycin which reaches the epithelium this has not been studied in the present investigation

Neomycin is known to have an inhibitory effect on conduction across myoneural junctions (Pittinger & Long, 1958 Füllman & Reuler 1960)

Wersäll & Flock (1964) have demonstrated that streptomycin, when applied locally in low concentrations to the lateral-line organ receptors, has a reversible effect on the sensory cells. However when a higher dose is given the sensory cells are destroyed and the effect irreversible.

In the experiment under review early changes were found in both the microvilli of the supporting cells and the kinocilium membrane of the sensory cells, when neomycin was applied locally. It seems reasonable to believe that some of these changes might be reversible. This seems likely especially as signs of the increased production of cilia, as indicated by the appearance of many basal bodies, in the upper parts of the sensory cells, were observed during neomycin treatment. The changes in the mitochondria and lysosomes are likely to be signs of more severe degeneration of the cell and might, in fact, be irreversible. Neomycin is known to disturb the protein synthesis of the cells partly by the destruction of the ribosomes. Some of th changes in the cytoplasm, with the appearance of large numbers of nongranulated vesicles, observed in the present study might extend to such changes in the epithelial cells.

It is i teresting to note that in those cases where severe changes were found in the membrane of the cilia, the fibers were still intact. This seems to indicate that a fibrillar portion of the cilia remains fairly stable despite the action of antibiotics. The changes in the vesicles of the cilia seem to be an early sign of a toxic effect. It is possible that the cells, in which this is the only morphological sign of toxicity may in fact be physiologically inacti e. This has to be proved, however by combined physiological and morphological studies similar to those made by Wersäll & Hawkins 1961) Wersäll & Flock (1964) and Duvall & Wersäll (1964) on the mechanoreceptor cells of the labyrinth.

The p esent work has demonstrated that neomycin is toxic to the olfactory mucosa both when given intraperitoneally and when applied locally. It strongly indicates that neomycin should not be applied in the nose so as to avoid toxic reactions in connection with the olfactory function.

ZUSAMMENFASSUNG

Die vorliegend Arbeit zeigt die toxische Wi kung vo Neomycin n ch sowohl lokaler al intraperitonealer Verabreichung. Die schwersten Veränderungen wurden in dem der intraperitonealen Zuführung am nächsten gelegenen T il des Epithel beoba htet, während die Veränderungen nach lokaler Zuführung mehr ausgebreitet, nahe der freien Oberflächen des Epith ls, waren. In den Stützzellen erscheint in Anwachsen der Zahl der Mitoch ndrien Lysosome doppelten Membranen Myelinfiguren und agranularen Vesikeln sowie in Verla gerung der Mikrovilli. Bei den Sinneszellen sieht man eine Degenera tion der Mitochondrien und äusseren Membranen und Vesikeln der Cilien sowie ine zunehmend Formation der Centri len. Die Bedeutung dieser Veränderungen für die Riecht aktion ist bereits diskutiert.

REFERENCES

DUVALL, A J and WERSÄLL, J 1964 Sit of action of trepl mycin upon inner ear sensory cells. *Acta Otolaryng* (Stockh) *57* 581

HAWKINS, J E., JR., 1959 Th otot xicity of kanamycin *Ann Otol 68* 698.

HAWKINS, J E., JR., and LURIE, M U 1952: The ototoxicity of streptomycin *Ann. Otol 61* 789

— 1953 The ototoxicity of dihydrostreptomycin nd neomycin in the cat *Ann. Otol., 62* 1128

KOHONEN A 1965 Effect of some totoxic drugs upon the pattern and innervation of ochl ar sensory c lls in the guinea pig *Acta Otolaryng* (Stockh.) S ppl 208 1

LUNDQUIST P-G and WERSÄLL, J 1966 Kanamycin induced changes in cochlear hair cells of the guinea pig *Z Z llforsch 72* 543

LÜLLM N U and REUTER, U 1960 Übe die H mmung d r neuromuskulären Übertragung durch einige Antibiotika *Chem therapia* (Basel) *1* 375

MILLONIG, G 1961 Advantages of a ph phat buffer from OsO solutio in fixation. *J Appl Physiol 32* 16

PITTINGER C. B and LONG, J P 1958 N uromuscular blocking action of neom cin. *Antibi t Chem th* (N Y) *8* 198

REYNOLDS, E. S 1963 The use of l ad itrat at high pH as an lectron st i in electron microsc py *J Cell Bi l 17* 208

SCHUKNECHT H F 1956 Ablati thera y f r th relief of M nière di ase *Laryngosc pe 66* 859

WATSON, M L., 1958 Staini g of tissue sections f lectron microscopy with hea y m tals *Biophy Biochem Cyt l* 4 475

WERSÄLL, J and FLOCK, A., 1964 S ppression and rest rati n of the micropho ic out put from the late al lin rg n fter loc l ppli ation of streptomyci *Life Sci nces 3* 1151

WERSÄLL, J nd H WKINS, J E., JR 1961: Th vestibula sensory epithelia i the cat labyrinth and their reacti ns in chronic trept mycin intoxic ti *A t Otolaryng* (Stockh.) 54 1

A Arstila M.D Dept of Anat my Univer ity of Turku Turku Finland
J W rsall M.D D pt of Otolaryngology Karolin ka Sjukh set Stockholm 60 Swed n

Received Janua y 9 1967

Acta oto-laryngologica 64, 313–316 (1967)

RECESSIVE EARLY-ONSET NEURAL DEAFNESS

M. C. Mengel, B. W. Konigsmark, C. I. Berlin and V. A. McKusick
Baltimore, Md. U.S.A.

From the Division of Medical Genetics, Department of Medicine and the Division of Laryngology and Otology, The Johns Hopkins University School of Medicine, Baltimore

In three generations of the S. family there were 16 members with severe deafness, all of whom were examined. By history all members were born with at least some hearing, with progressive severe loss by later childhood. Sonographic and speech analysis gave further evidence of at least some hearing in early childhood. Audiologic tests on 3 members of the family suggested a cochlear location of the defect. Other tests done on 10 affected members including physical, neurological, and vestibular examinations showed no abnormalities. The deafness appears to be transmitted as an autosomal recessive. We suggest that this entity be called "Recessive early-onset neural deafness".

In a recent review of the world's literature we have found about 54 different kinds of familial deafness in man. Most of these types of deafness are distinguished from the others by an association of other defects caused by the same gene such as albinism, nephritis, or goitre. However there remain a large group of hereditary deaf who, except for hearing loss, are normal in all regards.

We are studying families with congenital or early-onset severe deafness in order to define more clearly this heterogenous group of diseases. The present family came to our attention in a survey of severe deafness in Amish and Mennonite isolates. In three generations of the S. family there were 16 members with severe deafness of early onset.

SUBJECTS AND METHODS

The isolate

The Mennonites of Lancaster County, Pennsylvania, because of many doctrinal similarities to those of the Old Order Amish, have in general the same advantages for genetic study as do their more conservative Amish brethren. Unlike the Amish, the Mennonites are divided into several church

Supported in part by grant RD-1784-S-66 from the Vocational Rehabilitation Administration, Department of H. E. & W., The John A. Hartford Foundation, Inc., and NINDB Res. Career Dev. Award 5K3 NB, 19 488.

conferences, all with the same basic doctrine, but each with its own beliefs about dress, modes of transportation attitudes toward non Mennonites, etc. New converts are accepted but these are infrequent and usually have Amish or Mennonite ancestry They encourage marriage within their own conference and almost all Mennonites marry within their own faith

The S family

The S family is a part of the Mennonite isolate being studied for types of hereditary hearing loss. Local church and public school officials cooperating in pointing out deaf cases reported this family to us. The pedigree was completed by consulting family members, local historians, and published genealogies

All the affected living family members and most of their unaffected close relatives were visited a family and medical history were taken

Audiological testing

The audiological investigations were completed in 2 stages. One of us (M M) visited the communities with Beltone 10-C pure-tone audiometer and completed pure tone threshold on all affected family members These tests were done to calibration and response criteria used in the Hospital's Hearing and Speech Clinic. The completed audiograms were reviewed and those patients who had sufficient residual hearing to permit special tests for localization of auditory pathology received further study These included patients IX 3 X 6 7 and 8 (Fig 1)

The following tests were included in the diagnostic battery to help in assessing the locus of hearing loss

1 *Disyllabic word speech reception threshold* This test was used to support the validity of the pure-tone results since the ability to understand disyllabic words is correlated with the pure tone loss in the speech frequencies

2 *Bone conduction*

3 *Short increment sensitivity index (SISI)* An ability to detect a 1 dB increment 20 dB above threshold 90–100% of the time is defined as a positive SISI test and usually indicates cochlear pathology (Jerger Shedd & Harford 1959 Owens 1965)

4 *Tone decay* Though the principle underlying this test are unclear it has been observed that patients with retrocochlear lesions (ranging from acoustic neuromas, cerebral meningiomas to lues and Ramsey–Hunt Syndrome) usually show pathologic adaptation to a steady state tone (Owens, 1965)

Speech analysis

In order to reconstruct the early hearing history of members of the S family samples of speech of two members, K.S (VIII 2) and E.S (VIII-8)

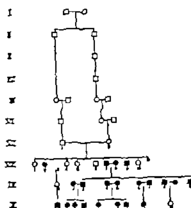


Fig. 1. The S. family pedigree showing 10 generations of the family. ■ deaf male; ● deaf female; □, hearing male; ○ hearing female; ◐ minimal hearing loss.

were subjected to sonographic analysis and speech analysis by skilled judges. The subjects read aloud a standard phonetic passage; this was recorded, and analysed by acoustic spectrography. A congenitally deaf patient, trained in oral school, spoke the same phrases for a control sonogram. The sonograms were analysed for characteristic durational qualities of the long-time deaf as compared to the congenitally deaf (Calvert, 1961). Five skilled listeners from the staff of the Hearing and Speech Clinic were then asked to judge the recordings according to whether they felt the subjects were essentially deaf since birth or at one time had some hearing. If they decided that the subjects were not deaf since birth the judges were asked to decide (*a*) whether there was a hearing loss, and (*b*) if so, at what age it might have begun to affect the speech monitoring of the speakers.

Additional examinations

Ten of the deaf members of the S. family had physical, neurological, and caloric vestibular examinations.

FINDINGS

History of early onset

By history the affected individuals had at least some hearing at birth, responding to sounds, and in some cases learning a few words. Progressive hearing loss occurred between the ages of 18 months and 6 years, with almost total deafness by 8 to 13 years of age. One child (X 9) was able to attend public school for four years before deafness forced her to attend a school for the deaf.

One would expect deaf parents to have difficulty evaluating the hearing

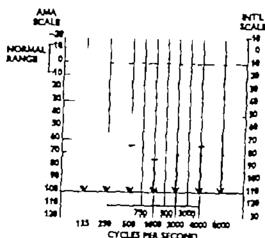


FIG 2. Hearing tests done on all affected S family members showed 80 dB to total hearing loss in all frequencies in this composite audiogram

status of their children but the history of an early onset, characteristic of this kindred has been witnessed by hearing neighbors and by one less severely affected member of the family Also the deaf parents related stories of their children hearing a barking dog or being able to tell their parents what was said on television It is also interesting that these deaf parents are able to tell approximately how severe each child's loss is, and which ear is worse The families in which the parents can hear likewise gave a history of early-onset rather than congenital deafness in their children

Though some of the affected have good speech this is not true for all, since they live in a world without audible language

Audiological findings

A typical audiogram (Fig 2) shows loss of 75–100 dB in all frequencies and demonstrates the range of hearing impairment in all affected family members

The audiograms of one family shown in Fig 3 show the similarities in close relatives The proband (IX-4) is a 42 year-old male who became deaf at 3–4 years of age His wife could hear and had some language before she became deaf at 2–3 years of age Of their four children, aged 5–10 years, three are deaf and according to the family the youngest hears some things though not as well as he once did This child is quite shy and could not be tested Contrary to what one might expect there is no progression in the severity of hearing loss from the youngest to the eldest child The most likely explanation is that a plateau of maximal hearing loss is reached at different ages. By history the age of onset ranges from 18 months in the youngest to 6 years in the eldest

The proband's brother and three children were studied in the Hearing and Speech Clinic The absence of any hearing by bone conduction indicates

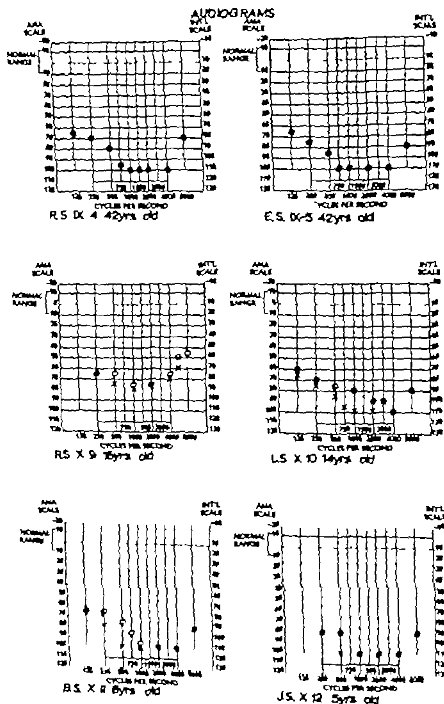


Fig. 3. Audiograms of the proband and his family. Bone conduction was absent in all tested. ●, right ear; X, left ear.

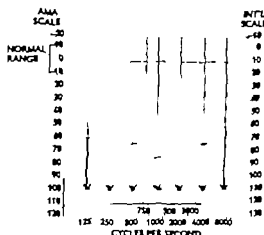


FIG 2 Hearing test done on all affected S family members showed 60 dB to total hearing loss in all frequencies in this composite audiogram.

status of their children, but the history of an early onset characteristic of this kindred has been witnessed by hearing neighbors and by one less severely affected member of the family Also the deaf parents related stories of their children hearing a barking dog or being able to tell their parents what was said on television It is also interesting that these deaf parents are able to tell approximately how severe each child s loss is, and which ear is worse The families in which the parents can hear likewise gave a history of early-onset rather than congenital, deafness in their children

Though some of the affected have good speech this is not true for all since they live in a world without audible language

Audiological findings

A typical audiogram (Fig 2) shows loss of 75–100 dB in all frequencies and demonstrates the range of hearing impairment in all affected family members

The audiograms of one family shown in Fig 3 show the similarities in close relatives. The proband (IX-4) is a 42 year-old male who became deaf at 3–4 years of age His wife could hear and had some language before she became deaf at 2–3 years of age Of their four children aged 5–16 years, three are deaf and according to the family the youngest hears some things, though not as well as he once did This child is quite shy and could not be tested Contrary to what one might expect there is no progression in the severity of hearing loss from the youngest to the eldest child The most likely explanation is that a plateau of maximal hearing loss is reached at different ages By history the age of onset ranges from 18 months in the youngest to 6 years in the eldest

The proband s brother and three children were studied in the Hearing and Speech Clinic The absence of any hearing by bone conduction indicates

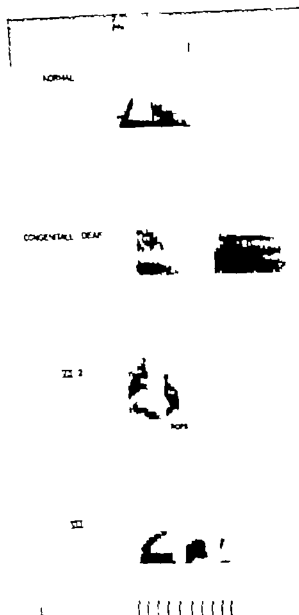


FIG. 4. Sonograms on the same t me base. These how the lmost normal word duration in the tw tested S. family members compared to the prolonged d ratio of words in congenitally deaf person.

history of grand mal epilepsy in several nieces and in the maternal grandmother of VII 2 (Fig. 1)

Transmission

The S. family pedigree (Fig. 1) show that J. S. (VII 1) and his second –'f (A. S. VII 2) are both descendants of Gl us Zimmerman (I 1) a

Act ala-l

a sensori neural loss. The two on whom a SISI could be done had a positive SISI score (90–100%) bilaterally indicating a cochlear origin of the loss.

Speech findings

Figure 4 shows the way in which a normal subject speaks the words rain drops" (an excerpt from a standard phonetic passage) The lower two sonograms show the same words spoken by two members of the S. family Note the lack of high frequency information in the upper portion of the spectrograms but the nearly normal vowel-consonant time relationships For comparison a congenitally deaf speaker trained in an oral school uttered the same words rain drops" Note the same absence of high frequency information but note also the extreme elongation of speech which is timed in msecs at the bottom of the figure Since the time values of English speech are carried in the low as well as the high frequency bands, we can say from these data that the speakers in the lower two sonograms must have had reasonably normal low tone hearing in their formative years.

The five skilled judges who were asked to assess the voice tape recording according to whether they felt the subjects were essentially deaf since birth or at one time had some hearing felt both speakers had severe hearing losses However both also were judged to have had good low tone hearing at least until their 5th to 7th years of life Two judges even picked out Amish or Germanic accents without any clues as to the birth place or religion of the speakers. These judgments were of course made without the judges knowing about the sonographic analysis.

Physical and neurological examinations

Physical examination showed normotensive individuals with normal ocular fundi and without cardiac respiratory or other abnormalities The cranial nerves were intact and there was no nystagmus Strength sensation reflexes and coordination were normal in all individuals tested There were no abnormalities of gait and Romberg tests were normal Vestibular testing by the Hallpike Cawthorn caloric method showed normal vestibular function

Mental development

The family is of normal intelligence functioning well in the community and doing semi skilled labor They have all attended the Pennsylvania School for the Deaf for periods of 3–12 years where they have done moderately well scholastically They communicated with us mostly by writing and were able to express themselves quite adequately in this way

Associated conditions

A family history taken from each adult family member showed no history of mental disease thyroid kidney nervous system or skin disorders, blindness, asthma allergy or physical deformities There is a

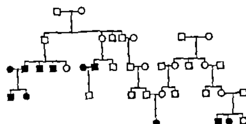


Fig. 5 Pedigree f Mrs. H S (indicated by arrow) IX-2 in Fig. 1 All of her 3 children are deaf

IX-6 is one of the least severely affected family members she has excellent speech She married a congenitally deaf male (IX 7) with no family history of deafness. They have a 9-year-old hearing daughter

IX-8 is a severely affected sib who became deaf at 3 years of age.

IX-9, th wife of IX-8 is congenitally deaf Their 6-month-old daughter (X 14) responds to sounds and turns to listen to a voice

IX 10 a brother became deaf at 2 years of age, had grandmal epilepsy and died at 20 years of age after hospitalization for seizures.

X 1 to X are normal hearing children

X-6 is a 10-year-old girl who has better hearing than either of her sibs. The parents cannot date the onset of her deafness. Her right ear has a 3-frequency a erage loss of 70 dB and her left 85 dB

X 7 is a 7 year-old girl who probably became deaf about 1 year of age She has a 3-frequency average loss of 90 dB.

X-8 is a 5-year-old boy who also became deaf about 1 year of age. He has a 3-frequency average loss of 90 dB

X 9 is a 17 year-old girl who became deaf gradually at the age of 5 or 6. She w s able to attend public school for four years bef re going to the Pennsylvania School for the Deaf Her audiogram is seen in Fig 2

X 10 i 16-year-old boy who became deaf at 4 years of age His audiogram is seen in Fig 3

X 11 is a 10-year-old girl who became deaf at 18 months of age. Her audiogram is seen in Fig 3.

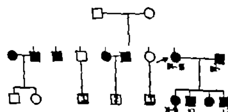


Fig. 6 The rrow indicates Mrs. R. S., IX-3 i Fig. 1 and wif f R. S. (IX 4) All of her 4 children are deaf

Mennonite immigrant who lived from 1647–1729 J S had seven normal children by his first wife, none of whom have deaf descendants. The pedigree (Fig 1) suggests autosomal recessive inheritance with a total of 9 males and 7 females affected

Case Reports

VII 1 (1842–1930) married S S and had 7 normal children After his first wife died he married A N (VII 2) and had 11 children one died in infancy and four were deaf

VIII 2, an aunt of the proband has excellent speech and a three frequency average (500 1000 and 2000 CPS) loss of 75 dB The age of onset is uncertain but the deafness seems to have progressed more slowly than in the rest A sonogram of her speech is shown in Fig 4

VIII-3 is another aunt with good speech and a three frequency average loss of 70 dB She married a male with normal hearing and has one daughter and 5 grandchildren all hearing

VIII 5 *is a female with normal hearing and a history of grandmal* epilepsy

VIII-6 *is the only hearing uncle to marry He has 11 normal* children and all normal grandchildren

VIII 7 the proband s father became deaf at 6 years of age and has a three frequency average loss of 95 dB

VIII-8 the proband s mother became deaf at 3–4 years of age She has 3 brothers 2 are normal and one became deaf at 4 years of age She has one nephew with a similar early-onset deafness Her parents had normal hearing A sonogram of her speech is shown in Fig 4

VIII 9 an uncle became deaf between 3 and 5 years of age and had a *history of grand mal epilepsy*

VIII 10 is a hearing uncle with grand mal epilepsy

VIII 11 died in infancy Hearing status is unknown

IX 2 is married to the probands brother IX-3 by whom she had 3 deaf children Her pedigree is shown in Fig 5 Her parents learned of her deafness when they took her to a physician at 1 year of age They are not certain whether she ever responded to sounds although they think she did

IX-3 the proband s brother became deaf at 4–5 years of age and remembers being able to hear with a hearing aid until the age of 9 when he lost interest in wearing it *He states that he could hear but did not learn* what the sounds meant as he had no need to develop language in his own environment Audiograms done when he was 13 years old show a three frequency average loss of 90 dB in the left ear and 100 dB in the right ear

IX-4 is the proband see text

IX-5 the proband s wife became deaf at 2–3 years of age Her pedigree is seen in Fig. 6 Her hearing parents state that all of their children responded to sounds and that some *had some language* when 4 *out of 6* became deaf at 1–4 years of age

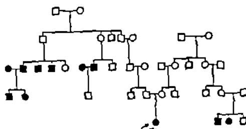


Fig. 3. Pedigree of Mrs. H. S. (indicated by arrow) IX 2 in Fig. 1. All of her 3 children are deaf

IX-6 is one of the least severely affected family members; she has excellent speech. She married a congenitally deaf male (IX 7) with no family history of deafness. They have a 9-year-old hearing daughter.

IX-8 is a severely affected sib who became deaf at 3 years of age.

IX 9, the wife of IX-8, is congenitally deaf. Their 6-month-old daughter (X 14) responds to sounds and turns to listen to a voice.

IX 10, a brother, became deaf at 2 years of age, had grandmal epilepsy and died at 20 years of age after hospitalization for seizures.

X 1 to X-5 are normal hearing children.

X-6 is a 10-year-old girl who has better hearing than either of her sibs. The parents cannot date the onset of her deafness. Her right ear has a 3-frequency average loss of 70 dB and her left, 85 dB.

X 7 is a 7 year-old girl who probably became deaf about 1 year of age. She has a 3-frequency average loss of 90 dB.

X-8 is a 5-year-old boy who also became deaf about 1 year of age. He has a 3-frequency average loss of 90 dB.

X 9 is a 17 year-old girl who became deaf gradually at the age of 5 or 6. She was able to attend public school for four years before going to the Pennsylvania School for the Deaf. Her audiogram is seen in Fig. 3.

X 10 is a 16-year-old boy who became deaf at 4 years of age. His audiogram is seen in Fig. 3.

X 11 is a 10-year-old girl who became deaf at 18 months of age. Her audiogram is seen in Fig. 3.

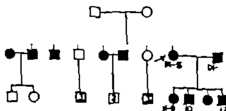


Fig. 4. The arrow indicates Mrs. R. S., IX-5 in Fig. 1, and wife of R. S. (IX-4). All of her 4 children are deaf

X 12 is a 5 year-old boy who still has some hearing by history We were unable to evaluate this because of his extreme shyness

X 13 is an 11 year-old girl who has had repeated bouts of tonsillitis and otitis media She had a 3-frequency average loss of 25 dB but had an upper respiratory infection each time she was tested She is unaware of any hearing loss and does well in public school

DISCUSSION

Etiology

Although there are many causes of deafness it seems unnecessary to invoke infection trauma, toxic drugs or noise as a cause of the deafness found in the S family There was no history in the affected members of any of these factors which might lead to deafness Physical examination showed normal tympanic membranes in all members of the family studied Neurological examination showed no abnormalities except for deafness. All affected members of the family had in fact a somewhat similar history of hearing loss with onset in early childhood and progression to a severe loss. Thus it seems most likely that the deafness in 16 members of this family is genetically determined

Mode of inheritance

Both males and females are affected in almost equal numbers (7 females and 9 males) making sex linked inheritance unlikely Although irregular dominant inheritance is a possibility in this pedigree the available evidence best supports recessive transmission The proband s father VIII 7 had 5 children all of whom were deaf His wife VIII-8, was also deaf Her hearing history is somewhat similar to that of the remainder of the S family She became deaf at 3–4 years of age In her family there is a history of deafness with involvement of 1 of her sibs and of a nephew Both of her parents had normal hearing Thus the history of deafness in the proband s mother s family is also most compatible with recessive transmission It is likely that her deafness was precisely the same as that of her husband, resulting in homozygous recessive deafness in all of their 5 children Case IX 3 also had a deaf wife IX 2 Although she had no sibs there is a history of deafness on both sides of her family but not in her parents. Two maternal second cousins were deaf Several paternal great cousins also were deaf as shown in the pedigree in Fig 5 Their 3 children X-6 7 and 8 are all deaf with a similar history of quite early onset The transmission of hearing loss in this family is quite compatible with recessive mode

The proband, IX-4 also married a woman with severe hearing loss Her pedigree is shown in Fig 6 Three of her 5 sibs are deaf Her normal hearing parents state that all of the children responded to sounds in early infancy Four of the 6 sibs became deaf from 1–4 years of age Both parents

had normal hearing and there was no past history of hearing loss in the entire family Again here hearing loss is most compatible with recessive transmission. Thus it is most likely that the mode of transmission of this familial deafness in the S. family is recessive

Time of onset

It appears that this is a progressive type of neural loss At the time of our pure-tone testing, all members tested had over a 60 dB loss in all frequencies tested. Although it is possible that the involved family members were born with this degree of hearing loss, there are two types of evidence which strongly suggest that the hearing loss was much less during infancy than it was at the time we were able to test any of the patients. The proband, IX-4 was quite sure that all of his 4 children had some hearing during infancy and that the hearing gradually worsened over the first few years of life. This observation was supported by the parents recall of in stances when the children reacted to sounds which the parents could not hear They even volunteered that there was some progression of hearing loss. A similar history was given by other parents in the family including the parents of the proband

More objective evidence is furnished that there was some residual hear ing in early childhood by the near-normal time relationships in the sonographic analysis of speech of se eral of the S. family members. As seen in the sonogram in Fig. 4 nd as also confirmed by the trained judges, speech of the two S. family members was m re like the normal than like a person who was born totally deaf It seems most reasonable from these two types of evidence, that the onset of de fness in the S. family was during early hildhood and that it wa not as severe at birth as it is some several years lat r

Location f lesion

It will be recalled that the air conduction losses exceeded 60 dB in the speech frequencies bone conduction showed no air-bone gap and the SISI test was positive in the 3 members of the family on whom this test could be done The tone decay test showed no significant decay thus suggesting no activ retrocochlear path fogy These result suggest the hearing loss is sensori-neural while the positive SISI test indicates a cochlear locus. No pathological studies are available.

Involvement of other ystems

Hallpike-Cawthorn caloric tests were don on 10 members of the family These sh wed a normal vestibular response with nyst gmus lasting from 1 2 minutes. There wa no spontaneous nystagmus on neurological exam ination

The Romberg test was normal in all cases Thus there is no evidence

X-12 is a 5-year-old boy who still has some hearing by history. We were unable to evaluate this because of his extreme shyness.

X-13 is an 11 year-old girl who has had repeated bouts of tonsillitis and otitis media. She had a 3-frequency average loss of 25 dB but had an upper respiratory infection each time she was tested. She is unaware of any hearing loss and does well in public school.

DISCUSSION

Etiology

Although there are many causes of deafness it seems unnecessary to invoke infection, trauma, toxic drugs or noise as a cause of the deafness found in the S. family. There was no history in the affected members of any of these factors which might lead to deafness. Physical examination showed normal tympanic membranes in all members of the family studied. Neurological examination showed no abnormalities except for deafness. All affected members of the family had, in fact, a somewhat similar history of hearing loss with onset in early childhood and progression to a severe loss. Thus it seems most likely that the deafness in 16 members of this family is genetically determined.

Mode of inheritance

Both males and females are affected in almost equal numbers (7 females and 9 males) making sex linked inheritance unlikely. Although irregular dominant inheritance is a possibility in this pedigree, the available evidence best supports recessive transmission. The proband's father, VIII-7, had 5 children, all of whom were deaf. His wife, VIII-8, was also deaf. Her hearing history is somewhat similar to that of the remainder of the S. family. She became deaf at 3–4 years of age. In her family there is a history of deafness with involvement of 1 of her sibs and of a nephew. Both of her parents had normal hearing. Thus the history of deafness in the proband's mother's family is also most compatible with recessive transmission. It is likely that her deafness was precisely the same as that of her husband, resulting in homozygous recessive deafness in all of their 5 children. Case IX-3 also had a deaf wife, IX-2. Although she had no sibs, there is a history of deafness on both sides of her family but not in her parents. Two maternal second cousins were deaf. Several paternal great cousins also were deaf as shown in the pedigree in Fig. 5. Their 3 children, X-6, 7 and 8, are all deaf with a similar history of quite early onset. The transmission of hearing loss in this family is quite compatible with recessive mode.

The proband, IX-4, also married a woman with severe hearing loss. Her pedigree is shown in Fig. 6. Three of her 5 sibs are deaf. Her normal hearing parents state that all of the children responded to sounds in early infancy. Four of the 6 sibs became deaf from 1–4 years of age. Both parents

the other family progressive hearing loss with onset from 2 to 16 years of age was described with progression to a moderate hearing loss. Cawthorne & Hinchliff (1957) presented 4 families with recessive inheritance of hearing loss. In two of these families an onset in early childhood was noted with progression to a moderate hearing loss. Although these families may be variants of the type of early onset neural deafness of the S. family it seems more likely because of the marked variation in the time of onset and the milder form of hearing loss, that they represent a different disease entity

Barr & Wedenberg (1965) presented a family in which 4 of 7 sibs had hearing impairments. They attended the school for the deaf in Stockholm. The entrance document stated that they heard and spoke a little, but not clearly Both parents had normal hearing. It seems quite possible that these families with an early onset of severe hearing loss, with recessive transmission, are afflicted by the same disease entity as that in the S family pedigree.

It is becoming clear as we continue on this project, that there are several types of hereditary deafness in man which need further clarification, since it is quite difficult to separate the varieties of deafness in this realm We have presented a family in which there is good evidence for recessive transmission of a genetic defect producing deafness There is also good evidence that the deafness is less severe during early infancy but that it reaches a plateau of marked severity in early childhood More examples of this type of familial deafness will clarify other parameters.

ZUSAMMENFASSUNG

Bei der Familie S. wurde innerhalb dreier Ge erationen bei 16 Familienangehörigen T ubheit festgest llt. All diese Menschen kon ten wi die Anamnese ergab, in den ersten Lebensjahren hören, we ch ingeschränkt. Im Laufe der iebsten Jahre wurd das Hörvermögen zunehmend schlechter Sonographie und Spra hanalyse gaben weitere Hinweise dafür dass in früher Kindheit geringes Hörvermögen besta den haben muss. Audiologisch U tersuchungen an dre Fam lienangehörigen weisen uf ine Def kt in der Regio ochlea hin. Andere U tersuchungen inschliesslich physikalischer eurologischer Unter suchungen und Prüfung des Vestibularapparates, zeigten k ine Abweichung von der Norm. Wahrschei lich wird die T ubh it durch einen rezessiven Erbfaktor hervorgerufen. Wir schlagen or dass dieses Krankh itsbild „recessive early onset neural deaf ess genannt wird.

REFERENCES

Albrecht W 1923 Über die Vererbung der konstit tion ll sporadischen T bstummheit, der hereditären Labyrinthschwerhörigkeit nd der Otosclerose Arch Ohr Nas. Kehlkopfheilk 110 15

of a vestibular apparatus defect in any of the cases. Physical and neurological examination showed no abnormalities A tomogram done on the proband showed no abnormality It would seem from these tests that the deafness is an isolated defect with no accompanying abnormalities in this family

Review of Literature

Although there are about 54 types of hereditary deafness distinguished in the literature only 9 of these have no associated abnormalities. These include Sex linked Congenital Severe Deafness described by Richards in 1963 "Sex linked Early-onset Neural Deafness" described by Mohr & Mageroy in 1960 Sex linked High Tone Neural Deafness" described by Livan (1961) Dominant Mid Frequency Neural Deafness" by Williams & Roblee (1962) Dominant Progressive Neural Deafness by Stephens & Dolowitz (1961) Dominant Unilateral Deafness" described by Smith (1939) and finally "Recessive Congenital Severe Deafness described by Fay in 1898 and more recently by Fraser (1964) It is fairly clear that the S family deafness is familial but not sexlinked The evidence is quite good that transmission is recessive rather than dominant

While dominant severe congenital deafness has been described by several authors including Stephenson & Cheeseman (1956) Chung, Robison & Morton (1959) Sank (1963) and Fraser (1964) only a few families with an early onset of dominant deafness have been described Albrecht (1923) presented several families with dominant progressive otosclerosis, clearly different from the S family s sensori neural hearing loss Although the dominant mid frequency neural deafness described by Williams & Roblee (1962) showed finally no evidence of progression of hearing loss in the mother and three children involved the mid frequency hearing loss described by Mårtensson (1960) gave evidence of progression in the six audiograms presented The dominant progressive deafness described by Stephens & Dolowitz (1961) was a slowly developing mild hearing loss in contrast to the rapid progression of the S family deafness Barr & Wedenberg (1965) presented an interesting pedigree one part of which shows a familial hearing loss of childhood onset with gradual progression to severe deafness by early adult life Transmission was apparently dominant with approximately half of the offspring of an affected parent developing hearing loss This may well be the same type of familial deafness as that described by Ford (1960) and by Huizing Van Bolhuis & Odenthal (1966) These families with their later onset and dominant transmission of hearing loss are quite different from the S family deafness

While numerous articles have appeared on recessive congenital profound deafness only a few papers described progressive deafness with recessive inheritance Johnson (1952) presented two families with recessive transmission of deafness. In one family the hearing loss was first noted at 10 years of age with progression to marked deafness by early adult life In

Acta oto-laryngologica 64 327–337 (1967)

ACTION OF STREPTOMYCIN DIHYDROSTREPTOMYCIN NEOMYCIN AND KANAMYCIN ON THE AMPULLAR RECEPTORS OF THE FROG

Y HARADA [1] E. MUSSO and E. MIRA
Pavia Italy

From the Institute of General Physiology (Head Prof C Casell) and from the Ot rhinolaryngological Clinic (Head Prof M Cherubin) University of Pavia

The action f several Strept myces ntibiotics on the ampullar receptors of the isolated posterior semicircular canal of the frog ha been studied using electrophysi logical methods. Both the ponta eou activity and the response t stimulus of the mpullar receptors are promptly and reversibly inhibited by the ntibi tics tested the depressa t action i higher for streptomy in and lower for dihydrostreptomycin neomycin and kanamy in

The peculiar action of the Streptomyces antibiotics on the vestibular and cochlear apparatus has been frequently established by clinical experience and pharmacological experimentation

Clinical observations concerned, first streptomycin and dihydrostreptomycin and m re recently neomycin and kanamycin (Hinshaw & Feldman 1945 B own & Hinshaw 1946 Carr *et al* 1950 *a* and *b* Bernard *t al.* 1950 Shane & Laurie 1950 Waisbren & Spink, 1951 Frost *et al* 1958–59 Lustberg & H mburger 19 9 Lecca *et al* 1950)

Wide electrophysiological experimentation has shown that both streptomycin and dihydrostreptomycin injected or locally applied in the bulla of the guinea-pig produce a strong depression of the cochlear microphonic potential and f the acoustic nerve impulses (Hawkins & Lurie, 1952–1953 Davi *et al* 1958 Tyberghein 1962 Ardouin *et al* 1963 Degreef 1964 Feinmesser & Sohmer 1965)

Much less numerou are the experimental data concerning the action of the above-mentioned antibiotics on the vestibular system

Changes in labyrinthine reflexes have been demonstrated by several authors in guinea pigs, r ts, cats and squirrel m nkeys treated with Strept myces antibiotics (Caussé *et al* 1948 Winston *et al* 1948, 1949 Ch istensen *et l* 1950 Berg, 1951 Rüedl *et al* 1952 Hawkins & Lurie 1953 Igarashi *t al* 1966) and Vallancien (1955) ha observed in frogs

This study wa pported by grant from the N tional Research Council (Electro-Physiological Enterprise) Rome, Italy

[1] Lecturer Otorhinolaryngologic l Cli ic of the U iversity of Hiroshima, J pan.

BARR, B and WEDENBERG, E., 1965 Prognosis of perceptive hearing loss i children with respect to g nesis and use of hearing aid Acta Otolaryng (Stockh.) 59 462.
CALVERT D R 1959: Dissertation Abstract 22 675
CAWTHOR E, T E., and HINCHLIFFE, R., 1957 Familial perceptive deafness Pract Oto-rhin laryng (Basel) 19 69
CHUNG, C. S ROBISON O W and MORTON, N E., 1959 A not n deaf mutism. Ann. Hum Genet 23 357
FAY E. A., 1898 Marriages of the Deaf in Ame ica Volta Bureau Washington
FELDMAN, A 1961 Problem in the measurement of Bone Conduction, J Speech Hearing Dis 26 39
FORD F R., 1960 Diseases of th Nervous System in Infancy Childhood and Adolescence p 410 Thomas, Springfield.
FRASER, G R 1964 Profound childhood deafness. J Med G n t 1 118.
HUIZING, E. H VAN BOLHUIS, A. H and ODENTHAL, D W 1966: Studies o progressive hereditary perceptive d afness in a family of 335 m mbers Acta Otolaryng (Stockh.) 61 35
JERGER, J SHEDD J L., and HARFORD, E., 1959: On the detection of extremely mall changes in Sou d Int nsity Arch Ot laryng (Chic.) 69 200
JOHNSEN, S 1952: The heredity of perceptiv deafness. Acta Otolaryng (Stockh.) 42 539
LINDENOV H 1945: The Etiology of Deaf Mutism with Special R ferences to Heredity Munksgaard, Copenhagen.
LIVAN M 1961 Contribute Alla Conoscenza Della Sordita Ereditarie Ar h Ital Otol 72 1
MARTENSSON, B 1960: Dominant hereditary n rve deafness. Acta Otolaryng (Stockh.) 5 2 0
MOHR, J and MAGEROY I 1960 Sex linked deafnes of a po sibly ew type Acta Genet 10 54
OWE s, E., 1965: Th SISI test nd VIIIth nerve versus cochl ar inv l em t. J Speech H aring Dis 30 252.
RICHARDS, B W 1963 Sex linked deaf mutism. Ann Hum G net 26 195
S NK, D 1963 Gen tic pect of early t tal d afness. In Family and M ntal Health Pr blem n a Deaf Population (ed J D Rainer K. Z. Alt huler and F J hall mann) p 28 New Y rk St te Psychiatric Institut N Y
SMITH, A B 1939 Unilat ral h reditary deaf es Lancet 237 1172.
STEPH s, F E., and DOLOWITZ, D A 1961 Heredit ry nerv deafness. Ann Otol # 851
STEVENSON A T d CHEESEM N E. A 1956 Hered tary de f m tism, with particul refere to N rthern Ir l nd Ann Hum Genet 20 177
WILLIAMS, F nd RO LEE, L. A 1962 Hereditary n rv d fnes A f ll w up f four cases in o f mil Arch Ot laryng (Chic) 7 69

B W K nig mark M.D Dept of Otol ryng logy Th J hn H pkins H pit l
Balt more 5 Md U.S A

Received February 1., 1967

Acta oto-laryngologica 61 387–397 (1967)

ACTION OF STREPTOMYCIN DIHYDROSTREPTOMYCIN NEOMYCIN AND KANAMYCIN ON THE AMPULLAR RECEPTORS OF THE FROG

Y Harada,[1] E. Musso and E. Mira
Pavia Italy

*From the Institute f General Physiology (Head Prof C Casella) and from the Otorhinolaryngological Clinic (Head Prof M Cherubin)
University of Pavi*

The act on of se eral Streptomyces antibiotics on the ampulla receptors of the isolated posterior semicircular canal of the frog has been studied using electrophysiological methods. Both the spontaneous activity and the response to stimulus of th mpulla receptors re promptly and reversibly inhibited by the antibiotics tested the depressant action is higher f r streptomycin and lower fo dihydrostrept mycin neomycin nd kanamycin.

The peculiar action of the Streptomyces antibiotics on the vestibular and cochlear apparatus has been frequently established by clinical experience and pharmacological experimentation.

Clinical observations concerned, first, streptomycin and dihydrostreptomycin and, more recently neomycin and kanamycin (Hinshaw & Feldman, 1945 Brown & Hinshaw 1946 Carr *et al* 1950 *a* and *b* Bernard *et al* 1950 Shane & Laurie 1950 Walsbren & Spink, 1951 Frost *et al* 1958–9 Lustberg & Hamburger 1959 Lecca *et al* 1959)

Wide electrophysiological experimentation has shown that both streptomycin and dihydrostreptomycin injected or locally applied in the bulla of the guinea-pig produce a strong depression of the cochlear microphonic potentials and of the acoustic nerve impulses (Hawkins & Lurie, 1952 1953 Davis *et al* 1958 Tyberghein 1962 Ardouin *et al* 1963 Degreef 1963 F lnmesser & Sohmer 1963)

Much less numerous are the experimental data concerning the action of the abov m ntioned antibiotics on the vestibul r system

Changes in labyrinthine reflexes have been demonstrated by several authors in guinea-pigs, rats, cats and squirrel monkeys treated with St eptomyces ntibiotics (Caussé *et al.*, 1948 Winst n *et al* 1948 1949 Christensen t al., 1950 Berg, 1951 Rüedl *et al* 1952 Hawkins & Lurie 1953 Igara hi *et al* 1960) nd Vallancien (1955) has observed in frogs

This study w supported by gr nt from the N tional Research Council (Electroph iological Enterprise) Roma, Italy

[1] Lecturer Otorhinol ryngological Cl ic of the University of Hiroshima, J pan.

injected with streptomycin a decreased afferent discharge in the ampullar nerves both during rest and after rotatory stimulation these toxic effects are evident however after a relatively prolonged administration of the antibiotics.

Of considerable interest are the observations of Wersall & Flock (1964) on the lateral line canal organ of fishes streptomycin, locally applied, even in high dilution (1/100 000) inhibits promptly the microphonic potentials, though inhibition is reversible with the withdrawal of the substance this pattern of the depressant action suggests that the antibiotic can directly interfere with the receptors mechanism

Histological investigations on the cochlear and vestibular apparatus of the laboratory animals have widely demonstrated that streptomycin and related antibiotics can produce elective lesions of the sensory cells (Wersäll & Hawkins, 1962 Duvall & Wersäll 1964 Spoendlin 1966)

The structural alterations may be related to the specific action of the Streptomyces antibiotics on cell membrane (McQuillen, 1951 Landman & Burchard 1962) or on the protein synthesis a curare-like action on cytoneural junctions is claimed by other authors (Brazil & Corrado 1957 Pittinger & Long 1958 Bezzi & Gessa 1959 Lullman & Reuter 1960 Osterloh 1961 Corrado & Nicoletti 1962 Kohonen 1965)

The problem of the mechanism of action of the antibiotics of the streptomycin group on the labyrinthine receptors is undoubtedly still open and data concerning the action of these antibiotics on the ampullar receptors appear at present, rather scanty

We therefore thought it would be interesting to undertake an electrophysiological study concerning the action of some antibiotics of the streptomycin group on the ampullar receptors of the frog

An experimental condition suitable for a quantitative evaluation of the activity of streptomycin dihydrostreptomycin neomycin and kanamycin has been achieved by employing an isolated semicircular canal preparation and by counting the action potentials in the ampullar nerve fibres under adequate stimulation of the crista ampullaris.

METHOD

The dissection and isolation of the posterior semicircular canal of the frog (*Rana esculenta*) has been described in a previous paper (Rapuzzi 1966) The canal is isolated together with the corresponding ampullar branch of the vestibular nerve and the preparation is dipped in a Petri dish filled with Tyrode solution

The tip of a thin glass pipette is introduced under microscope control in the cutted posterior end of the isolated canal The pipette is connected with a micrometer syringe filled with Tyrode solution By rotating the micrometer syringe a few degrees a controlled flow of fluid in the canal and in the ampulla has been obtained (Fig 1)

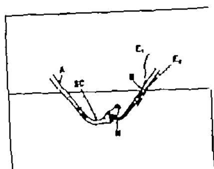


Fig. 1

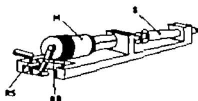


Fig. 2.

Fig. 1. Semi-schematic drawing f the preparation *SC*, isolated semicircula canal *Y* ampullar nerv *A B* gl ss pipettes; E_1, E_2, leading-off lectrodes.

Fig. 2. Stim lating device: *RS*, releasing screw *RB*, rubber ba d; *M* micrometer; *S* syringe.

A suitable rubber-band operating device allows a carefully controlled rotation of the micrometer screw (5 degrees in 0 1 sec) corresponding to an ampullofugal displacement of a 0 15 μl of fluid in the isolated canal this has been used as standard stimulus for the preparation (Fig. 2)

The action potentials in the ampullar branch of the vestibular nerve were led-off by means of a second pipette provided with two platinum electrodes the cutted end of the branch was sucked in the opening of the pipette acting as a gap between two fluid electrodes (Rapuzzi & Casella 1965)

Both the stimulation and leading-off devices were handled by means the two arms of a micromanipulator

The action potentials, conventionally amplified, were [illegible] CRO and counted by means of a scaling unit

Th response to the stimulation has been evaluated [illegible] number of action potentials discharged within 7 sec [illegible] application.

The pontaneou activity of the ampullar recept [illegible] count f the action potentials just before the sti[illegible] t th response to th stimulus.

The action of the tested substances has bee[illegible] th response to th timul s before and after t[illegible] fluid, accord ng to the f llowing sequence [illegible]

(1) The a ti ity of the preparati n a [illegible] stimulation were determined 20 minutes af[illegible]

(2) Measurements were repeated ever[illegible] biotic to the bathing fluid.

(3) After 42 minutes the preparation [illegible] repeatedly [illegible] solution in order to remove the antib[illegible] and th [illegible] mination were repeated at 6-minut interval [illegible] minutes.

injected with streptomycin a decreased afferent discharge in the ampullar nerves both during rest and after rotatory stimulation these toxic effects are evident however after a relatively prolonged administration of the antibiotics

Of considerable interest are the observations of Wersäll & Flock (1964) on the lateral line canal organ of fishes streptomycin, locally applied even in high dilution (1/100 000) inhibits promptly the microphonic potentials, though inhibition is reversible with the withdrawal of the substance this pattern of the depressant action suggests that the antibiotic can directly interfere with the receptors mechanism

Histological investigations on the cochlear and vestibular apparatus of the laboratory animals have widely demonstrated that streptomycin and related antibiotics can produce elective lesions of the sensory cells (Wersäll & Hawkins, 1962 Duvall & Wersäll 1964 Spoendlin, 1966)

The structural alterations may be related to the specific action of the Streptomyces antibiotics on cell membrane (McQuillen 1951 Landman & Burchard, 1962) or on the protein synthesis a curare-like action on cytoneural junctions is claimed by other authors (Brazil & Corrado, 1957 Pittinger & Long 1958 Bezzi & Gessa, 1959 Lullman & Reuter 1960 Osterloh 1961 Corrado & Nicoletti 1962 Kohonen 1965)

The problem of the mechanism of action of the antibiotics of the streptomycin group on the labyrinthine receptors is undoubtedly still open and data concerning the action of these antibiotics on the ampullar receptors appear at present rather scanty

We therefore thought it would be interesting to undertake an electrophysiological study concerning the action of some antibiotics of the streptomycin group on the ampullar receptors of the frog

An experimental condition suitable for a quantitative evaluation of the activity of streptomycin dihydrostreptomycin, neomycin and kanamycin has been achieved by employing an isolated semicircular canal preparation and by counting the action potentials in the ampullar nerve fibres under adequate stimulation of the crista ampullaris.

METHOD

The dissection and isolation of the posterior semicircular canal of the frog (*Rana esculenta*) has been described in a previous paper (Rapuzzi, 1966) The canal is isolated together with the corresponding ampullar branch of the vestibular nerve and the preparation is dipped in a Petri dish filled with Tyrode solution

The tip of a thin glass pipette is introduced under microscope control in the cutted posterior end of the isolated canal The pipette is connected with a micrometer syringe filled with Tyrode solution By rotating the micrometer syringe a few degrees a controlled flow of fluid in the canal and in the ampulla has been obtained (Fig 1)

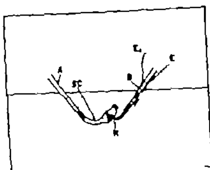


Fig. 1.

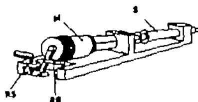


Fig. 2.

Fig. 1 Semi-schematic drawing of the preparation *SC*, isol ted semicircular canal; N ampullar nerv *A B*, glass pipettes; E_1, E_2 leading-off lectrodes.

Fig. 2 Stimulating de ice *RS*, releasi g screw *RB* rubber ba d; *M* micrometer *S* syringe.

A suitable rubber-band operating device allows a carefully controlled rotation of the micrometer screw (3 degrees in 0 1 sec) corresponding to an ampullofugal displacement of a 0 15 μl of fluid in the isolated canal this has been used as standard stimulus for the preparation (Fig 2)

The action potentials in the ampullar branch of the vestibular nerve were led-off by means of a second pipette provided with two platinum electrodes the cutted end of the branch was sucked in the opening of the pipette acting as a gap between two fluid electrodes (Rapuzzi & Casella, 196.)

Both the stimulation and leading-off devices were handled by means of the two arms of a micromanipulator

The action potentials, conventionally amplified, were monitored on a CRO and counted by means of a scaling unit.

The response to the stimulation has been evaluated by counting the number of action potentials discharged within 7 sec after the stimulus pplication.

The spontaneous activity f the ampullar receptors was estimated by a count of th action potentials just before the stimulation and subtracted t the response to the stimulus.

The action of the tested substances has been evaluated by comparing the response to the stimulus before and after their addition to the bathing fluid, according to the following sequence of measurements

(1 Th ctivity of the preparation t rest and the response to the stimulation were determined 20 minutes after the dissection

(2 Mea urement w re repeated every 6 minutes after adding the anti biotic to the bathing fluid

(3) After 42 minutes the preparation wa repeatedly washed with Tyrode solu tion in order to remove th antibiotic and the abov mentioned deter mination were repeated at 6-minute intervals for a t tal period of 42 minutes.

injected with streptomycin a decreased afferent discharge in the ampullar nerves both during rest and after rotatory stimulation these toxic effects are evident, however after a relatively prolonged administration of the antibiotics

Of considerable interest are the observations of Wersall & Flock (1964) on the lateral line canal organ of fishes streptomycin, locally applied, even in high dilution (1/100 000) inhibits promptly the microphonic potentials, though inhibition is reversible with the withdrawal of the substance this pattern of the depressant action suggests that the antibiotic can directly interfere with the receptors mechanism

Histological investigations on the cochlear and vestibular apparatus of the laboratory animals have widely demonstrated that streptomycin and related antibiotics can produce elective lesions of the sensory cells (Wersäll & Hawkins, 1962 Duvall & Wersall 1964 Spoendlin 1966)

The structural alterations may be related to the specific action of the Streptomyces antibiotics on cell membrane (McQuillen 1951 Landman & Burchard 1962) or on the protein synthesis a curare-like action on cytoneural junctions is claimed by other authors (Brazil & Corrado 1957 Pittinger & Long, 1958 Bezzi & Gessa, 1959 Lullman & Reuter 1960 Osterloh, 1961 Corrado & Nicoletti 1962 Kohonen 1965)

The problem of the mechanism of action of the antibiotics of the streptomycin group on the labyrinthine receptors is undoubtedly still open and data concerning the action of these antibiotics on the ampullar receptors appear at present rather scanty

We therefore thought it would be interesting to undertake an electrophysiological study concerning the action of some antibiotics of the streptomycin group on the ampullar receptors of the frog

An experimental condition suitable for a quantitative evaluation of the activity of streptomycin, dihydrostreptomycin, neomycin and kanamycin has been achieved by employing an isolated semicircular canal preparation and by counting the action potentials in the ampullar nerve fibres under adequate stimulation of the crista ampullaris.

METHOD

The dissection and isolation of the posterior semicircular canal of the frog (*Rana esculenta*) has been described in a previous paper (Rapuzzi 1966) The canal is isolated together with the corresponding ampullar branch of the vestibular nerve and the preparation is dipped in a Petri dish filled with Tyrode solution

The tip of a thin glass pipette is introduced under microscope control in the cutted posterior end of the isolated canal The pipette is connected with a micrometer syringe filled with Tyrode solution By rotating the micrometer syringe a few degrees a controlled flow of fluid in the canal and in the ampulla has been obtained (Fig 1)



Graph 1



Graph 2.

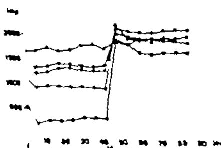
Graph 1. Action of streptomycin on the ampullar receptors' response to stimulation. Addition (') and removal (") of the antibiotic. ▲—▲—▲ control, ■—■—■ streptomycin 3.2 10^{-4} M ●—●—● streptomycin 6.4 10^{-4} M ○—○—○ streptomycin 1.28 10^{-4} M □—□—□ streptomycin 3.2 10^{-4} M

Graph 2. Action of dihydrostreptomycin on the ampullar receptors response to stimulation. Addition (') and removal (") of the antibiotic. ▲—▲—▲ control, ●—●—● dihydrostreptomycin 6.4 10^{-4} M ○—○—○ dihydrostreptomycin 1.28 10^{-4} M □—□—□ dihydrostreptomycin 3.2 10^{-4} M

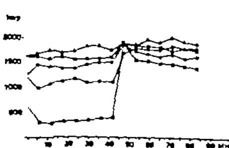
Spontaneous activity during treatment of the preparation is depressed also by dihydrostreptomycin and enhanced after removal of the substance. In this case too, however, dihydrostreptomycin appears to be less active than streptomycin.

Neomycin

As appears from Graph 3 and Fig. 3, this substance is even less active than dihydrostreptomycin, the inhibiting concentration ranging between



Graph 3



Graph 4

Graph 3. Action of neomycin on the ampullar receptors response to stimulation. Addition (') and removal (") of the antibiotic. ▲— — control; ■—■—■ neomycin 1.6 10^{-4} M ●— — neomycin 3.2 10^{-4} M ○—○—○ neomycin 4.8 10^{-4} M □—□—□ neomycin 10 M

Graph 4. Action of kanamycin on the ampullar receptors response to stimulation. Addition (') and removal (") of the antibiotic. ▲— — control ■—■—■ kanamycin 1.6 10^{-4} M — —● kanamycin 3.2 10^{-4} M ○— —○ kanamycin 4.8 10^{-4} M □—□—□ kanamycin 8.0 10^{-4} M

Measured amounts of the antibiotics were dissolved in a small volume of Tyrode solution added to the bathing fluid in order to obtain the following final concentrations

Streptomycin sulphate 3.2×10^{-5} M 6.4×10^{-5} M 1.28×10^{-4} M 3.2×10^{-4} M
Dihydrostreptomycin sulphate 3.2×10^{-5} M 6.4×10^{-5} M 1.28×10^{-4} M 3.2×10^{-4} M
Neomycin sulphate 1.6×10^{-4} M 3.2×10^{-4} M 4.8×10^{-4} M 8.0×10^{-4} M
Kanamycin sulphate 1.6×10^{-4} M 3.2×10^{-4} M 4.8×10^{-4} M 8.0×10^{-4} M

Not less than five experiments have been performed on different preparations for every tested concentration of each substance. Control experiments have been performed for each group of treatments in plain Tyrode solution

RESULTS

Graphs 1–4 have been traced with the data obtained from the various group of experiments the amplitude of the response to the stimulus before during and after the action of the antibiotics tested has been mapped during the course of the experiment

Streptomycin

As appears from Graph 1 and from the Fig 3 this antibiotic has an evident depressant action on the ampullar response to the stimulus even at very low concentration

The inhibition to the response is slight at 3.2×10^{-5} M concentration and increases with increasing concentration(s) The response to the stimulus is completely abolished at a concentration of 3.2×10^{-4} M

It has been noted that in every case the action of streptomycin was established immediately after the application of the substance and is considerably reversed by washing the preparation

The spontaneous activity of the preparation is also influenced by streptomycin which exerts an evident depressant action at concentrations between 10^{-5} and 10^{-4} M After removal of the antibiotic and washing of the preparation the spontaneous activity appears notably enhanced for a period of about 5 minutes (Graph 5)

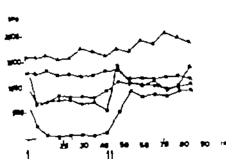
Dihydrostreptomycin

The results obtained are reported in Graph 2

Dihydrostreptomycin similarly to streptomycin, depresses the ampullar receptors response at low concentration (Fig 3)

The observed inhibition is significantly lower than that of the streptomycin The graph shows that a 6.4×10^{-5} M solution is completely ineffective and that a complete suppression of the response could not be obtained even with solutions containing 3.2×10^{-4} M of the antibiotic

Dihydrostreptomycin appears to act less promptly than streptomycin its action is, however easily reversed by washing the preparation





Graph 1 Graph 2.

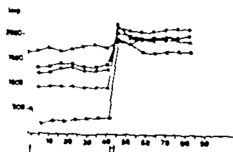
Graph 1 Action of streptomycin on the ampullar receptors response to stimulation. Addition (') and removal (") of the antibiotic ▲—▲—▲ control, ■—■—■ streptomycin 3.2 10^{-4} M ●— —● streptomycin 6.4 10^{-4} M ○—○—○ streptomycin 1.28 10^{-3} M □—□—□ streptomycin 3.2 10^{-4} M

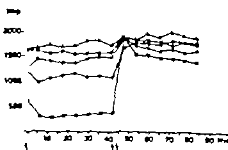
Graph 2. Action of dihydrostreptomycin on the ampullar receptors response to stimulation. Addition (') and removal (") of the antibiotic. ▲—▲—▲ control, ●—●—● dihydrostreptomycin 6.4 10^{-4} M —○— dihydrostreptomycin 1.28 10^{-3} M □—□—□ dihydrostreptomycin 3.2 10^{-4} M

Spontaneous activity during treatment of the preparation is depressed also by dihydrostreptomycin and enhanced after removal of the substance in this case too, however dihydrostreptomycin appears to be less active than streptomycin

Neomycin

As appears from Graph 3 and Fig. 3, this substance is even less active than dihydrostreptomycin, the inhibiting concentration ranging between

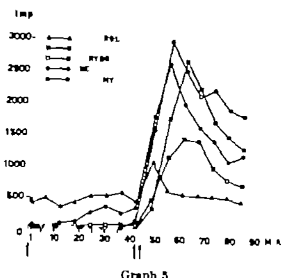




Graph 3 Graph 4.

Graph 3 Action of neomycin on the ampullar receptors response to stimulation. Addition (') and removal (") of the antibiotic — —▲ control ■—■—■ neomycin 1.6 10^{-4} M — —● neomycin 3.2 10^{-4} M — — neomycin 4.8 10^{-4} M — — neomycin 1 M

Graph 4 Action of kanamycin on the ampullar receptors response to stimulation. Addition (') and removal (") of the antibiotic ▲— —▲ control, ■—■—■ kanamycin 1.6 10^{-4} M; ●—●—● kanamycin 3.2 10^{-4} M ○— — kanamycin 4.8 10^{-4} M □—□—□ kanamycin 8.0 10^{-4} M



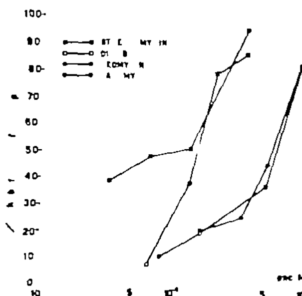
Graph 5

Graph 5 Action of the antibiotics, added at the highest concentration (streptomycin and dihydrostreptomycin 3.2×10^{-4} M neomycin and kanamycin 8.0×10^{-4} M) on the spontaneous activity of the ampullar receptors. Addition (↑) and removal (↑↑) of the antibiotics.

10^{-4} and 10^{-3} M The depressant action is easily and completely reversible even at the higher concentrations employed Neomycin depresses, too, the spontaneous activity which is enhanced after removal of the substance and washing of the preparation (Graph 5)

Kanamycin

Kanamycin (Graph 4) acts very similarly to neomycin both concerning the modalities and the intensity of its action on the ampullar response to



Graph 6 Depressant action of the antibiotics on the response of the ampullar receptors to stimulation as a function of their concentration. The inhibition is expressed as a percent decrease of the original response

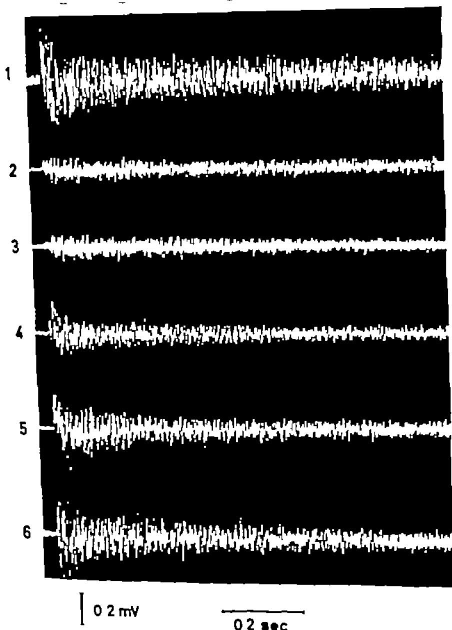
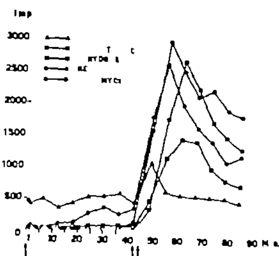


Fig. 2. Response to stimulation of the ampullar receptors of the isolated posterior semicircular canal (1) before adding the antibiotics (2) 15' after administration of streptomycin 6.4 $10^{[illegible]}$ M; (3) dihydrostreptomycin 1.28 10^{-4} M (4) neomycin 8.0 $10^{[illegible]}$ M; (5) kanamycin 8.0 10^{-4} M and (6) 5' after removing the antibiotics.

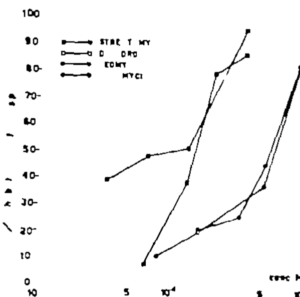


Graph 5 Action of the antibiotics, added at the highest concentration (streptomycin and dihydrostreptomycin 3.2 × 10⁻⁴ M neomycin and kanamycin 8 0 × 10⁻⁴ M) on the spontaneous activity of the ampullar receptors. Addition (↑) and removal (↑↑) of the antibiotics.

10^{-4} and 10^{-3} M The depressant action is easily and completely reversible even at the higher concentrations employed Neomycin depresses, too, the spontaneous activity which is enhanced after removal of the substance and washing of the preparation (Graph 5)

Kanamycin

Kanamycin (Graph 4) acts very similarly to neomycin both concerning the modalities and the intensity of its action on the ampullar response to



Graph 6 Depressa t cti n f th ntibiotics th response of the mp llar recept rs to stim lati n as a functi n f th i concentrati The inhibiti i xpressed a a perc nt decrease of the original respo se

man 1965) and of those reported by Voldrich (1965) and by Stupp *et al.* (1966) in the internal ear fluids of experimentally-treated guinea-pigs.

The inhibitory action of streptomycin and related antibiotics on the ampullar sensory apparatus appears to be very prompt and fairly reversible in our experimental records: this observation suggests that antibiotics act on rather superficial structures of the labyrinthine sensorial apparatus and that their bonds with the substratum are at least initially not very deep.

The site of action of the antibiotics as suggested by Duvall & Wersäll (1964) could be at the sensorial cells surface, where they can easily come diffusing through the cupula or the subcupular space (Dohlman, 1960).

The possibility that the antibiotics may interfere with the transmission at the cytoneural junction cannot, however, be discounted.

Further experimentation is still necessary before any definitive conclusion concerning the mechanism of action of the antibiotics on the labyrinthine apparatus can be drawn.

RÉSUMÉ

On a étudié chez la grenouille l'action de plusieurs substances antibiotiques du groupe de la streptomycine sur les récepteurs ampullaires du canal semicirculaire postérieur isolé. Les antibiotiques examinés exercent une inhibition prompte et réversible soit sur l'activité des récepteurs ampullaires à répos, soit sur leur réponse à la stimulation. L'inhibition est plus marquée pour la streptomycine et moindre pour la dihydro-streptomycine, la néomycine et la kanamycine.

ZUSAMMENFASSUNG

Die Wirkung verschiedener Streptomyces-Antibiotika auf die ampullaren Rezeptoren des isolierten hinteren Bogengangs des Frosches wurde an Hand von elektrophysiologischen Methoden beobachtet. Sowohl die spontane Funktion als auch die Reaktion der ampullären Empfangsapparate auf Reizeinwirkung wird prompt und reversibel von den geprüften Antibiotika inhibiert, bei Streptomycin ist die deprimierende Wirkung maximal, bei Dihydrostreptomycin, Neomycin und Kanamycin niedriger. Die Bedeutung dieser Ergebnisse wird diskutiert.

REFERENCES

Arnoux, P., Suter, L. and Johano, P. 1963. Étude électro-physiologique de l'otolithe de certains antibiotiques. *Acta Otolaryng. (Stockh.)* 56, 106.

Berg, K. 1951. The toxic effect of streptomycin on the vestibular and cochlear apparatus. *Acta Otolaryng. (Stockh.)* Suppl. 97.

Br[illegible]n, E., Paul, P. V. and A[illegible]n, G. 1950. Sur l'action cochléovestibulaire de la dihydrostreptomycine. *Bull. Mém. Soc. Méd. Hôp. Paris* 66, 383.

[illegible]an, G. and Green, O. L. 1959. Neuromuscular blocking action of some antibiotics. *Nature* 184, 903.

Brazil, O. V. and Corrado, A. P. 1957. The curariform action of streptomycin. *J. Pharm.* 120, 452.

the stimulus The depression of the receptor s response produced by this antibiotic, however appears to be less reversible than that of the former drugs

The spontaneous activity was depressed by kanamycin only at the higher concentrations Enhancement of the spontaneous activity was observed after washing of the preparation

In Graph 6 the inhibition of the ampullar response to the stimulus is represented as a function of the concentration of the different antibiotics tested the data refer to measurements taken at the 18th minute after the application of the substance to the preparation i e when the action of the antibiotic is at its apex.

It clearly appears that streptomycin is the antibiotic more intensely depressant to the ampullar receptors

DISCUSSION

The results obtained in this research prove that all the antibiotics of the Streptomyces group can strongly influence *in vitro* the function of the ampullar sensory apparatus of the frog

Even at very low concentrations they produce an evident depression both of the response to stimulation and of the spontaneous discharge observed in the resting preparation

The action of the antibiotics is very prompt and easily reversible after their removal from the bathing solution

Some differences, however in the action of the four antibiotics tested have been noted

Streptomycin appears to be the most active depressant of the receptor s response to stimulation and of the spontaneous discharge The other antibiotics appear in general less active specially on the spontaneous activity of the preparation Kanamycin seems to exert an action more difficult to reverse

The results are mainly in agreement with the clinical observations concerning the higher labyrinthine toxicity of streptomycin in respect to the other antibiotics of the same group (Tyberghein, 1962 Feinmesser & Sohmer 1965)

The agreement between the *in vivo* and *in vitro* observations concerning the intensity of action on the labyrinthine receptors suggests that the differences noted between the single antibiotics are mainly due to the peculiar pharmacological activities of the different molecules on the receptors and cannot be ascribed as sometimes is claimed to their different passage through the labyrinthine barrier

It is, however to be noted that the antibiotic concentrations we found active *in vitro* are of the same order of magnitude as those normally reached in human fluids during therapeutic treatment (Goodman & Gil

man, 1963) and of those reported by Voldrich (1965) and by Stupp *et al.* (1966) in the internal ear fluids of experimentally treated guinea pigs.

The inhibitory action of streptomycin and related antibiotics on the ampullar sensory apparatus appears to be very prompt and fairly reversible in our experimental records: this observation suggests that antibiotics act on rather superficial structures of the labyrinthine sensorial apparatus and that their bonds with the substratum are, at least initially, not very deep.

The site of action of the antibiotics, as suggested by Duvall & Wersäll (1964) could be at the sensorial cells surface, where they can easily come diffusing through the cupula or the subcupular space (Dohlman, 1960).

The possibility that the antibiotics may interfere with the transmission at the cytoneural junction cannot, however, be discounted.

Further experimentation is still necessary before any definitive conclusion concerning the mechanism of action of the antibiotics on the labyrinthine apparatus can be drawn.

RÉSUMÉ

On a étudié chez la grenouille l'action de plusieurs substances antibiotiques du groupe de la streptomycine sur les récepteurs ampullaires du canal semicirculaire postérieur isolé. Les antibiotiques examinés exercent une inhibition prompte et reversible soit sur l'activité des récepteurs ampullaires à répos, soit sur leur réponse à la stimulation. L'inhibition est plus marquée pour la streptomycine et moindre pour la dihydro-streptomycine, la néomycine et la kanamycine.

ZUSAMMENFASSUNG

Die Wirkung verschiedener Streptomyces-Antibiotika auf die ampullären Rezeptoren des isolierten hinteren Bogenganges des Frosches wurde an Hand von elektrophysiologischen Methoden beobachtet. Sowohl die spontane Funktion als auch die Reaktion der ampullären Empfangsapparate auf Reizeinwirkung wird prompt und reversibel von den geprüften Antibiotika inhibiert; bei Streptomycin ist die deprimierende Wirkung maximal, bei Dihydrostreptomycin, Neomycin und Kanamycin niedriger. Die Bedeutung dieser Ergebnisse wird diskutiert.

REFERENCES

Andoire, P., Sarr, L. and Jourdan, P. 1963. Étude électro-physiologique de l'ototoxicité de certains antibiotiques. *Acta Otolaryng* (Stockh.) *56*, 108.

Berg, K. 1951. The toxic effect of streptomycin on the vestibular and cochlear apparatus. *Acta Otolaryng* (Stockh.) Suppl. 97.

Bravard, E., Paar, P. Y. and Aran, C. 1950. L'action cochléo-vestibulaire de la dihydrostreptomycine. *Bull. Mém. Soc. Méd. Hôp.* Paris, *66*, 583.

Brazil, O. and Grass, O. L. 1959. Neuromuscular blocking action of some antibiotics. *Nature 183*, 983.

Brazil, O. V. and Corrado, A. P. 1957. The curariform action of streptomycin. *J. Pharm. 120*, 452.

Brown H. A and Hinshaw H C., 1946 Toxic reaction of streptomycin on the eighth nerve apparatus. *Proc. Staff Meet May Clinic 21* 347

Carr, D T Brown, H A Hodgson L. H and Heilman F R 1950 Neurotoxic reactions to dihydrostreptomycin *J.A.M.A* 143 1223

Carr, T C., Brown H A and Pfuetze, K H 1950 Occurrence of deafness in neomycin therapy *J A M.A* 144 65

Caussé, R Gondet I and Vallancien B 1948 Acti n vestibulaire de la streptom cine chez la souris. *C.R Soc. Bi l* (Paris) 14 747

Christensen E., Hertz, H Riskaer, N nd Vra -Jensen G 1950: Experiments on the neurotoxic effect of streptomycin *Acta Otolaryng* (Stockh.) Suppl 95 163.

Corrado A P and Nicoletti, A R 1962 Mechanism of neuromuscular block produced by streptomycin, dihydrostreptomycin, neomycin and kanamycin. *Acta Fisiologica Latino Am icana 12* 212.

Davis, H Deatherage, B H Rose blut B Fernández, C., Kimura, C., and Smith, C A 1958 Modificati n of cochle r potentials produced by streptomycin poisoning nd by xtensive venou obstru tion *Laryngoscope 68* 596

Deorreo I 1964: The ot toxicity f kanamycin A theoretical and xperiment l study *Acta Ot hin laryng B lg* 18 415

Dohlman, G F 1960 Histochemic l studies of vestibula mechani m In *Neural me hani ms of auditory and ve tibula sy tems* (ed. by C Rasmu sen nd O Windle) Cha les C. Th ma Publ Springfield, Ill

Duvall, A J nd Wersäll, J 1964 Site of action of strept mycin upo t her ca sensory ells. *Acta Otolaryng* (Stockh.) 7 581

Friedmann, M nd Sohmer, H 1963: Influ nce f streptomy in and dihydrostreptomycin on th cochlear pot nti l f th guin a pig *Ann Otol* 72 48

Frost J O Daly J F nd Hawk s, J E., Jr 1958/59: The ot t icity of kanamycin in man *Antibiot Ann* 700

Goodman L. S and Gilman A 1965 *The pharmacolog al basi f therapeutics* 3rd ed. McMillan Co New Y rk

Hawkins, J E., Jr nd L rie, M H 19 2 Th ot t xicity of trept mycin. *An Otol* 61 789

— 1953: The otot xicity f dihydro trept my i and eomycin in the c t *An Otol* 62 1128

Hinshaw H C., nd Feldm W H 1945 Strept m ci in treatment of li ical tubercul l *P oc Staff M t Mayo Cl ni* 20 313

Kohonen A 1965: Effect of some t t xic drug po the pattern a d i nerv tion of coc lea sory cell in th guin a pig *A ta Ot laryng* (Stockh) Suppl 208

Ioana hi, M McLeod, M F and Graybiel, A 1966 Cl ni l pathological correl tion in squirr l m k ft suppressi n f semic rcul can l f ction by trept m in sulph t *A ta Ot la yng* (Stockh) S ppl 14

Landm O E and B rch rd, W 1962 Th mecha i m f ti n f trept m in revealed b n rmal nd bnormal di l l n n trept m n-d pe dent salm ll *Proc N t Acad Sci U.S.A* 48 210

Lecca, C G T rr J M oriolo L., d Mo les, A 19 0 Ot t xicity f kanam i *J.A M.A* 1 0 2064

Lullm n, H nd Re ter, H 1960 Über di Hemm g d euromu kulären Übertragung durch Inig A tibi tika *Ch m t apl* (Basel) 1 373

L ustberg, A and Hamberger, M 1959 Eighth rv d afnes fte admi i trati f k namycin. *J A.M.A* 1 0 606

McQ ille K 1951 Effect f trept m in o the lectrophoretic m bility f Escherichia coli and Staph lococcu reus. *Biochim Bi phy A ta* 7 54

Otterlob G 1961: N urom kul Eff kt ba ischer Streptom ces-A tibiotika und deren Aufhebung d rch Calcium. *Arzneimitt If s hung 11* 1130

Pittman, C. B., and Long, J. P. 1958. Neuromuscular blocking action of neomycin. Ant biol. Chem. th ... 8 198.
Rossi, G., 1966. La ma ifesta ... Jettriche d l recettori ampollari nei canali semi-circolari isolati. *Boll. Soc. Ital. Biol. Sper.* In press.
Rossi, G., and Casella, C., 1965. I nerv tion f the fungiform papilla i the frog tongue. *J. N rophysiol.* 28, 154.
Rüedi, L., F anz, W., Lutz, F. Nager, C., and Tschirren, B., 1952. Further observations concerning the t ic ffect f strept my in nd q i ine on the ditory rga of guinea-pigs. *Laryngoscope* 62, 333.
Shane, S. J. nd La am, J. H., 1950. Neurot to icity of dihydrostreptomycin. *Canad. Med. Ass. J.* 63, 277.
Spoendlin, H. 1964. Z Otot iritat des Streptomyzins. *Pract. Otorhinolaryng.* (Basel) 26, 305.
Stupp, H., Ra n, S. So s, H. nd Lagler, F. 1966. Untersuchungen über die Ursache der perif ch ot ischen Wirk g der ba ischen Strept myces-A tibiotika ter besonderer Berücksichtigung des Kanamy ins. *Act Otolaryng.* (Stockh.) 61, 435.
Tyberghein, J. 1962. I fl ence f me streptomyces antibiotics on the cochlear microphonics i the guinea pig. *Act Otolaryng.* (Stockh.) Suppl. 171.
Vilstrup, B., 1955. *Effets de qu lques t iques ur les pot tials d'action d estibule de la grenouill. Pract. Ot rhinolaryng.* (Basel) 17, 157.
Voldrich, L. 1965. The ki etics f streptomy in, kanamy i and neomyci i th inner ar. *Act Otol ryng.* (Stockh. 60, 243.
Warren, B. A. nd Serva, W. W. 1954. A linical ppraisal of neomy i. *Ann. I t rn. Med.* 33, 1099.
Wersäll, J. 1958. St dies the struct re nd innerv tion of the sensory pithelium of the cristae ampullares i the guinea pig. *Act Otolaryng.* (Stockh.) Suppl. 126.
Wersäll, J. nd Flock, A., 1964. S ppre on d restoration of the microphonic output from the lateral line org after local pplic tion of streptomy in. *Lif Sci.* 3, 1151.
Wersäll, J. nd Haw ins, J. E., Jr., 1962. The estibular sensory epithelia i the cat labyrinth d thei reactions i cronic strept my i toxicati n. *A t Otolaryng.* Stockh. 54, 1.
Winston, J. Lewey, F. H. P rkinson, A. Marden, P. A. nd Cramer, F. B., 1948. An experiment l study of the t ic effect of streptom ci the estibular pparat of the cat. Part I the central nervou ystem. *Ann. Otol.* 57, 738.
1949. Further experimental studies f the t ic ffect of treptomyci on central estibular pparat f the cat. *A n. Otol.* 58, 988.

D. E. M re. Ot hinolaryngological Clinic
University f Pavia, Pavia, Italy

Rece ved F b uary 8, 1967

Acta oto-laryngologica 64 338–344 (1967)

UNTERSUCHUNGEN ZUR 1. PERROTATORISCHEN NYSTAGMUSPERIODE

R. GROHMANN und B. MINNIGERODE

Göttingen, Deutschland

Aus der Universitäts-Hals-Nasen-Ohrenklinik (Direktor: Prof. Dr. A. Miehlke) Göttingen

Nachdem in zwei früheren Mitteilungen über die Altersabhängigkeit der Gesamtamplituden und Frequenzen von zeitlich begrenzten Nystagmusperioden berichtet wurde und die Einordnung dieser für den Nystagmusablauf spezifischen Charakteristika in das Nystagmusgeschehen erfolgte, wurden nunmehr spezielle Untersuchungen zur 1. perrotatorischen Nystagmusperiode durchgeführt. Diese bei Beschleunigung einer Versuchsperson einsetzende Nystagmusperiode wird in eine Beschleunigungs- und perrotatorische Abkling-Nystagmusperiode unterteilt. Sowohl die Gesamtamplituden und Frequenzen dieser Teilabschnitte der 1. perrotatorischen Nystagmusperiode als auch die der Beschleunigungs-Nystagmusperiode vorausgehende Latenzzeit wurden auf ihre Abhängigkeit vom Alter geprüft. Die Besonderheiten im Verhalten der Gesamtamplituden und der Frequenzen der Beschleunigungs- und der perrotatorischen Abkling-Nystagmusperiode sowie der der erstgenannten Nystagmusperiode vorausgehenden Latenzzeit, für die alle eine eindeutige Altersabhängigkeit nachgewiesen werden konnte, werden aufgezeigt. Der im Gegensatz zu der perrotatorischen Abkling-Nystagmusperiode steilere altersbedingte Frequenzanstieg und Gesamtamplitudenabfall in der Beschleunigungs-Nystagmusperiode läßt auf eine mit dem Alter zunehmende Verschlechterung der Fähigkeit schließen, einen äußeren physikalischen Reiz in einen inneren physiologischen zu transformieren.

Vorbemerkung

In früheren Untersuchungen zur Altersabhängigkeit von Amplitude und Frequenz (Minnigerode u. Grohmann 1966, Minnigerode, Grohmann u. Vontin 1967, Grohmann u. Minnigerode 1967) wurde die 1. perrotatorische Nystagmusperiode als der vom Einsetzen des Nystagmus nach Beginn der Drehbeschleunigung bis zu seinem Wiederverschwinden nach dem Beschleunigungsende sichtbare Reizeffekt des Vorhofbogengangsapparates definiert, wobei unberücksichtigt blieb, daß die Drehbeschleunigung schon während dieser Nystagmusperiode endet, der Nystagmus aber darüber hinaus noch einige Zeit andauert. Zur eingehenderen Analyse des Ablaufes der 1. perrotatorischen Nystagmusperiode haben wir daher im folgenden den Zeitraum der durch die Endolymphströmung hervorgerufenen Cupula-Auslenkung von dem ihrer noch während der Drehung erfolgenden Rückkehr in die Ausgangslage getrennt. Es ergibt sich daraus

vom nystagmographischen Standpunkt her die Aufgliederung in eine *Beschleunigungs-Nystagmusperiode* die vom Einsetzen des Nystagmus nach Beginn der Drehbeschleunigung von 3 /sec^2 bis zum Beschleunigungsende (= Übergang in eine gleichmäßige Winkelgeschwindigkeit von 90 /sec) anzusetzen ist und in eine perrotatorische *Abkling-Nystagmusperiode* die vom Beschleunigungsende bis zum Verschwinden des Nystagmus, d. h. bis zu Nystagmusschlägen von weniger als 0,5 Winkelgrad, andauert. Da die 1. perrotatorische Nystagmusperiode nicht unmittelbar mit dem Beginn der Drehbeschleunigung, sondern erst nach einer bestimmten Zeit, der sogenannten *Latenzzeit* meist mit Nystagmusschlägen geringer Intensität und Frequenz einsetzt, bildete diese ebenso wie die genannten beiden Teilabschnitte der 1. perrotatorischen Nystagmusperiode sowie ihre Abhängigkeit vom Alter das Ziel der vorliegenden Untersuchungen. Die Methodik ihrer experimentellen Erforschung und anschließenden Auswertung entsprach dabei unter Zugrundelegen derselben Versuchspersonen den an anderer Stelle ausführlich beschriebenen Einzelheiten (Minnigerode u. Grohmann 1966).

Latenzzeit

Das schwingungsfähige System dessen Funktion vom erfaßbaren Nystagmus angezeigt wird, reagiert erst nach einigen Sekunden auf einen äußeren Reiz, solange dieser eine bestimmte obere Grenze nicht überschreitet. Diese sogenannte Latenzzeit kann sowohl bei der quantitativen thermischen Erregbarkeitsprüfung nach Frenzel (1959) als auch bei der überschwelligen Dreherregbarkeitsprüfung der Labyrinthe beobachtet werden. Wird eine gesunde Versuchsperson z. B. mit 3 /sec^2 auf einem Drehstuhl bis zu einer Winkelgeschwindigkeit von 90 /sec beschleunigt und aus dieser nach einigen Minuten mit 3 /sec verzögert, so leitet die Latenzzeit die oben definierte Beschleunigungs-Nystagmusperiode und bei Verzögerung die entsprechend festzusetzende, dann einen Teil der 1. postrotatorischen Nystagmusperiode bildende Verzögerungs-Nystagmusperiode ein. Stoppt man dagegen den Drehstuhl aus der Winkelgeschwindigkeit von 90 /sec plötzlich ab, so beginnt die 1. postrotatorische Nystagmusperiode sofort und besteht nicht wie bei der Verzögerung aus Verzögerungs- und postrotatorischer Abkling-Nystagmusperiode, sondern nur aus der letzteren. Eine Latenzzeit besteht bei diesem sehr starken Reiz nicht mehr.

Ilnur u. Mendel (1960) beschrieben kürzlich eine deutliche Relation zwischen der Dauer der Latenzzeit und der Stärke des äußeren Reizes. Wie zu erwarten ergab sich eine Verkürzung der Latenzzeit mit Zunahme der Drehreizstärke. Obwohl nicht ausdrücklich betont, läßt ihre Mitteilung dennoch erkennen, daß eine mögliche Abhängigkeit der Latenzzeitdauer vom Alter der Probanden unberücksichtigt blieb. Da aber bei einer Beschleunigung von 3 /sec^2 nachweislich eine Altersabhängigkeit der Latenzzeit besteht und nicht vorausbestimmt werden kann, wie sich diese Abhängigkeit vom Alter bei einer Variation der Reizstärken auswirkt,

Acta oto-laryngologica 64 333-344 (1967)

UNTERSUCHUNGEN ZUR 1. PERROTATORISCHEN NYSTAGMUSPERIODE

R. GROHMANN und B. MINNIGERODE
Göttingen, Deutschland

Aus der Universitäts-Hals-Nasen-Ohrenklinik (Direktor: Prof. Dr. A. Miehlke) Göttingen

Nachdem in zwei früheren Mitteilungen über die Altersabhängigkeit der Gesamtamplituden *und Frequenzen von zeitlich begrenzten Nystagmus*perioden berichtet wurde und die Einordnung dieser für den Nystagmusablauf spezifischen Charakteristika in das Nystagmusgeschehen erfolgte, wurden nunmehr spezielle Untersuchungen zur 1. perrotatorischen Nystagmusperiode durchgeführt. Diese bei Beschleunigung einer Versuchsperson einsetzende Nystagmusperiode wird in eine Beschleunigungs- und perrotatorische Abkling-Nystagmusperiode unterteilt. Sowohl die *Gesamtamplituden und Frequenzen dieser Teilabschnitte der 1.* perrotatorischen Nystagmusperiode als auch die der Beschleunigungs-Nystagmusperiode vorausgehende Latenzzeit wurden auf ihre Abhängigkeit vom Alter geprüft. Die Besonderheiten im Verhalten der Gesamtamplituden und der Frequenzen der Beschleunigungs- und der perrotatorischen Abkling-Nystagmusperiode sowie der der erstgenannten Nystagmusperiode vorausgehenden Latenzzeit, für die alle eine eindeutige Altersabhängigkeit *nachgewiesen werden konnte, werden aufgezeigt.* Der im Gegensatz zu der perrotatorischen Abkling-Nystagmusperiode steilere altersbedingte *Frequenzanstieg und Gesamtamplitudenabfall in* der Beschleunigungs-Nystagmusperiode läßt auf eine mit dem Alter zunehmende Verschlechterung der Fähigkeit schließen, einen äußeren physikalischen Reiz in einen inneren physiologischen zu transformieren.

Vorbemerkung

In früheren *Untersuchungen zur Altersabhängigkeit von Amplitude und* Frequenz (Minnigerode u. Grohmann 1966, Minnigerode, Grohmann u. Vontin, 1966; Grohmann u. Minnigerode, 1967) wurde die 1. perrotatorische Nystagmusperiode als der vom Einsetzen des Nystagmus nach Beginn der Drehbeschleunigung bis zu seinem Wiederverschwinden nach dem Beschleunigungsende sichtbare *Reizeffekt des Vorhofbogengangapparates* definiert, wobei unberücksichtigt blieb, daß die Drehbeschleunigung schon während dieser Nystagmusperiode endet, der Nystagmus aber *darüber* hinaus noch einige Zeit andauert. Zur eingehenderen Analyse des Ablaufes der 1. perrotatorischen Nystagmusperiode haben wir daher im folgenden den Zeitraum *der durch die Endolymphströmung hervorgeru*fenen Cupula-Auslenkung von dem ihrer noch während der Drehung erfolgenden Rückkehr in die Ausgangslage getrennt. Es ergibt sich daraus

wirkung von Endolymphströmung (nystagmusfördernd) und Deformation bzw Verschiebung des häutigen Bogenganges (nystagmushemmend) auf die Labyrinthrezeptoren. Eine solche Annahme setzt aber gerade hinsichtlich der Deformations- bzw Verschiebungskomponente starke äußere Reize voraus, bei denen jedoch erfahrungsgemäß keine oder nur eine sehr kurze Latenzzeit beobachtet werden kann. Sehr viel wahrscheinlicher und mit den Untersuchungsergebnissen von Fluur u Mendel (1966) in Einklang zu bringen ist es, daß die Cupula eine bestimmte Druckreizschwelle besitzt, deren Erreichung bzw Überschreitung für die Dauer der Latenzzeit verantwortlich ist.

Mit Hilfe der Latenzzeit und den zwischen den per und postrotatorischen Nystagmusperioden eingeschalteten Pausen wurde nochmals versucht, eine einzige charakteristische Größe für den experimentellen Nystagmus abzuleiten Eine multiplikative Verknüpfung der Latenzzeit und der Pausendauer mit den Gesamtamplitudenwerten oder den reziproken Frequenzwerten der vorausgegangenen oder auch nachfolgenden Nystagmusperioden führte ebenso wie die Zusammenfassung mit den Werten des Produktes A F zu keiner weiteren Vereinfachung in der Beschreibung des Nystagmusgeschehens. Es ergibt sich daraus erneut, daß der experimentelle Nystagmus bei Betrachtung vollständiger Nystagmusperioden nicht mit einer einzigen Größe beschrieben werden kann Es bestätigt sich dagegen unsere frühere Feststellung, daß die Angabe der Amplitude und der Frequenz zur Beschreibung der einzelnen Nystagmusperioden notwendig ist und für die klinischen Belange auch ausreicht Bei detaillierten Untersuchungen erscheint es aber zweckmäßig, die 1 perrotatorische und bei Verzögerung auch die 1 postrotatorische Nystagmusperiode in eine perrotatorische Beschleunigungs- und Abkling-Nystagmusperiode bzw postrotatorische Verzögerungs- und Abkling Nystagmusperiode zu zerlegen und die der Beschleunigungs- bzw auch Verzögerungs-Nystagmusperiode vorausgehende Latenzzeit zu berücksichtigen

Gesamtamplitude und Frequenz der perrotatorischen Beschleunigungs und Abkling-Nystagmusperiode

Die Unterteilung der 1 perrotatorischen Nystagmusperiode in eine Beschleunigungs- und eine Abkling-Nystagmusperiode führt zu den Abbildungen 2 und 3. In diesen Abbildungen sind die mittleren Gesamtamplituden und Frequenzen pro Altersgruppe die sich als arithmetische Mittelwerte der individuellen Gesamtamplituden und Frequenzen aller einer Altersgruppe angehörenden Versuchspersonen ergeben, für die Beschleunigungs- und Abkling-Nystagmusperiode der 1 perrotatorischen Nystagmusperiode dargestellt Die Summe der Gesamtamplituden dieser beiden Teilabschnitt der perrotatorischen Nystagmusperiode ergibt zwangsläufig die Gesamtamplitude der nicht unterteilten 1 perrotatorischen Nystagmusperiode Für die Frequenzen gilt dies aber nicht, weil sie aus Schlagzahl

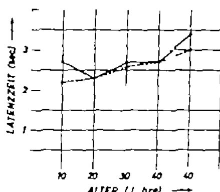


Abb. 1 Altersabhängigkeit der gemittelten, der Beschleunigungs-Nystagmusperiode vorausgehenden Latenzzeit ——— Rechtsdrehung - - - Linksdrehung

müßte der von ihnen angedeutete funktionelle Zusammenhang zwischen Latenzzeit und Reizstärke für verschiedene Altersgruppen überprüft werden bevor er in der von Fluur u Mendel (1966) mitgeteilten Form als funktions-diagnostischer Bestandteil in die Beurteilung des Elektronystagmogramms eingehen kann

Abbildung 1 zeigt die Abhängigkeit der gemittelten der Beschleunigungs Nystagmusperiode vorausgehenden Latenzzeit vom Alter Die Kurven für Rechts- und Linksdrehung lassen regelmäßig eine Zunahme der Latenzzeit mit steigendem Lebensalter erkennen Eine altersbedingte Erhöhung der Empfindlichkeitsschwelle des Systems wie sie bereits in den einzelnen Nystagmusperioden bei Amplitude und Frequenz zum Ausdruck kam kann auch als Ursache für die Verlangerung der Latenzzeit mit zunehmendem Alter angesehen werden

Neben der Latenzzeit wurde auch die Altersabhangigkeit der Dauer der zwischen den 1 und 2 perrotatorischen sowie 1 und 2 postrotatorischen Nystagmusperioden zu beobachtenden Pausen untersucht Die Dauer dieser Pausen zeigte jedoch bei Betrachtung von Rechts und Linksdrehung keine eindeutige Beziehung zum Alter Von einer graphischen Darstellung wurde daher abgesehen Sehr wahrscheinlich werden diese Pausen nicht nur von den unmittelbar vorangegangenen Nystagmusperioden beeinflußt sondern auch von dem Vermogen des Zentrums, diese Reize zu kompensieren

Als Ursache fur das Auftreten einer Latenzzeit bei nicht zu starken auf das Vestibularissystem einwirkenden Reizen konnen strömungsphysikalische elektrochemische und nervose Vorgange angesehen werden Das Verschwinden der Latenzzeit bei sehr starken Reizen macht es wahrscheinlich daß den stromungsphysikalischen Faktoren die möglicherweise die Dampfung des schwingungsfähigen Systems mitbedingen oder sie beeinflussen ein bestimmender Einfluß auf die Latenzzeit zukommt und daß diese somit ausschließlich Teil der peripher ausgelosten Vorgange des experimentellen Nystagmus ist Szentágothai (1952) diskutiert in diesem Zusammenhange in Anlehnung an Lorente de Nó (1928) die Bedeutung der im Bereich der horizontalen Bogengange gegenteilig arbeitenden Ein

wirkung von Endolymphströmung (nystagmusfördernd) und Deformation bzw. Verschiebung des häutigen Bogenganges (nystagmushemmend) auf die Labyrinthrezeptoren. Eine solche Annahme setzt aber gerade hinsichtlich der Deformations- bzw. Verschiebungskomponente starke äußere Reize voraus, bei denen jedoch erfahrungsgemäß keine oder nur eine sehr kurze Latenzzeit beobachtet werden kann. Sehr viel wahrscheinlicher und mit den Untersuchungsergebnissen von Pfaltz u. Mendel (1960) in Einklang zu bringen ist es, daß die Cupula eine bestimmte Druckreizschwelle besitzt, deren Erreichung bzw. Überschreitung für die Dauer der Latenzzeit verantwortlich ist.

Mit Hilfe der Latenzzeit und den zwischen den per- und postrotatorischen Nystagmusperioden eingeschalteten Pausen wurde nochmals versucht, eine einzige charakteristische Größe für den experimentellen Nystagmus abzuleiten. Eine multiplikative Verknüpfung der Latenzzeit und der Pausendauer mit den Gesamtamplitudenwerten oder den reziproken Frequenzwerten der vorausgegangenen oder auch nachfolgenden Nystagmusperioden führte ebenso wie die Zusammenfassung mit den Werten des Produktes $A \times F$ zu keiner weiteren Vereinfachung in der Beschreibung des Nystagmusgeschehens. Es ergibt sich daraus erneut, daß der experimentelle Nystagmus bei Betrachtung vollständiger Nystagmusperioden nicht mit einer einzigen Größe beschrieben werden kann. Es bestätigt sich dagegen unsere frühere Feststellung, daß die Angabe der Amplitude und der Frequenz zur Beschreibung der einzelnen Nystagmusperioden notwendig ist und für die klinischen Belange auch ausreicht. Bei detaillierten Untersuchungen erscheint es aber zweckmäßig, die 1. perrotatorische und bei Verzögerung auch die 1. postrotatorische Nystagmusperiode in eine perrotatorische Beschleunigungs- und Abkling-Nystagmusperiode bzw. postrotatorische Verzögerungs- und Abkling-Nystagmusperiode zu zerlegen und die der Beschleunigungs- bzw. auch Verzögerungs-Nystagmusperiode vorausgehende Latenzzeit zu berücksichtigen.

Gesamtamplitude und Frequenz der perrotatorischen Beschleunigungs- und Abkling-Nystagmusperiode

Die Unterteilung der 1. perrotatorischen Nystagmusperiode in eine Beschleunigungs- und eine Abkling-Nystagmusperiode führt zu den Abbildungen 2 und 3. In diesen Abbildungen sind die mittleren Gesamtamplituden und Frequenzen pro Altersgruppe, die sich als arithmetische Mittelwert der individuellen Gesamtamplituden und Frequenzen aller einer Altersgruppe angehörenden Versuchspersonen ergeben, für die Beschleunigungs- und Abkling-Nystagmusperiode der 1. perrotatorischen Nystagmusperiode dargestellt. Die Summe der Gesamtamplituden dieser beiden Teilabschnitte der perrotatorischen Nystagmusperiode ergibt zwangsläufig die Gesamtamplitude der nicht unterteilten 1. perrotatorischen Nystagmusperiode. Für die Frequenzen gilt dies aber nicht, weil sie aus Schlagzahl

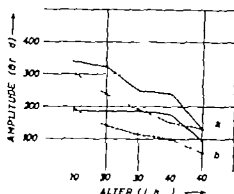


Abb 2.

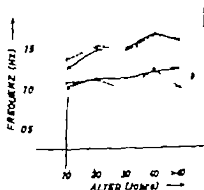


Abb 3

ABB 2. Verh lten der Gesamtamplitude (Mitt lwertskurven) d r perrotat rischen Beschleunigungs- (*a*) und Abkling Nystagmusperiode (*b*) in verschiedenen Altersgruppen. Erläuterung im Text ——— Rechtsdrehung - - - Linksdrehung

ABB. 3 Frequ nzverhalt n (Mittelwertskurven) d r perrot t rischen Beschleu igungs- (*a*) und Abkling Nystagmusperiod (*b*) in verschiedenen Altersgruppen. Erläuterung im Text ——— Rechtsdrehung - - - Li ksdrehung

und Periodendauer getrennt für die Beschleunigungs und die zugehörige Abkling Nystagmusperiode berechnet wurden Die Frequenzen der nicht unterteilten 1 perrotatorischen Nystagmusperiode liegen ungefähr in der Mitte zwischen den Frequenzen der Beschleunigungs und der Abkling Nystagmusperioden

Aus Abbildung 2 wird ersichtlich daß bei einer Drehstuhlbeschleunigung von 3 /sec² die bis zum Erreichen der Winkelgeschwindigkeit von 90 /sec dauert die Gesamtamplitude der Beschleunigungs Nystagmusperiode in allen Altersgruppen größer als die der perrotatorischen Abkling-Nystagmusperiode ist und mit dem Alter stärker absinkt Die Mittelwertskurven der Abbildung 3 zeigen das Frequenzverhalten unter gleichen Bedingungen Die Frequenz der Beschleunigungs-Nystagmusperiode liegt dabei in allen Altersgruppen hoher und nimmt mit dem Alter starker zu als die der perrotatorischen Abkling Nystagmusperiode wobei der Anstieg in der Beschleunigungs Nystagmusperiode großer in der Abkling Nystagmusperiode bei Berücksichtigung der Frequenz für die Linksdrehung jedoch etwas kleiner als in der nicht unterteilten 1 perrotatorischen Nystagmusperiode ist Während die Teilamplituden der Beschleunigungs und perrotatorischen Abkling Nystagmusperiode zwar verschieden stark mit dem Alter fallen fallt doch jede für sich genommen weniger als die Gesamtamplitude der nicht unterteilten 1 perrotatorischen Nystagmusperiode Ebenso wie die Amplituden selbst addieren sich die Neigungen der altersabhängigen Teilamplitudenkurven der Beschleunigungs- und perrotatorischen Abkling Nystagmusperiode zum Anstieg der Gesamtamplitudenmittelwertskurve der nicht unterteilten 1 perrotatorischen Nystagmusperiode

Bei einer Winkelbeschleunigung von 3 /sec² wächst die Rotations

geschwindigkeit des Drehstuhles linear von 0 /sec bis 90 /sec in 30 sec. Berücksichtigt man, daß die Latenzzeit mit zunehmendem Alter im Mittel von etwa 2,5 sec auf ungefähr 3,2 sec ansteigt so ergibt sich eine mittlere Dauer der Beschleunigungs-Nystagmusperiode von 27,5 sec für jüngere und 26,8 sec für ältere gesunde Versuchspersonen

Ebenso wie die 1 postrotatorische Nystagmusperiode die nach dem plötzlichen Stop einer mit konstanter Winkelgeschwindigkeit gedrehten Versuchsperson einsetzt, können die Abkling-Nystagmusperioden, die nach Beschleunigungs oder Verzögerungsende beginnen, als freie Schwingungen eines gedämpften schwingungsfähigen Systems (Mittermaier 1939 Grohmann u. Minnigerode 1967) beschrieben werden Der geringe Frequenzanstieg und Gesamtamplitudenabfall in Abhängigkeit vom Alter kann in diesen Perioden auf einen altersbedingten Elastizitätsverlust des schwingungsfähigen Systems zurückgeführt werden Die im Verhältnis zur Frequenz der Beschleunigungs-Nystagmusperiode deutlich geringere Frequenz in der perrotatorischen Abkling Nystagmusperiode bestätigt die an anderer Stelle gezogene Schlußfolgerung (Grohmann u Minnigerode 1967) daß sich die Frequenz der 1 perrotatorischen Nystagmusperiode am Ende der Periode der niedrigeren Eigenfrequenz des Systems, mit der die Nachreaktionen ablaufen, annähert. Dabei ist zu berücksichtigen, daß die in der Abbildung 3 dargestellten Frequenzen der perrotatorischen Abkling Nystagmusperiode Mittelwerte über die unterschiedlichen Frequenzen dieser Periode sind und daher keine Aussagen über die Frequenzen zu Beginn und am Ende der Abkling Nystagmusperiode zulassen.

Die Beschleunigungs- und Verzögerungs-Nystagmusperioden stellen erzwungene Schwingungen dar deren hohe Amplituden und Frequenzen vorwiegend v m äußeren Reiz bestimmt werden Der im Gegensatz zu der perrot t rischen Abkling Nystagmusperiode teilere altersbedingte Frequenzanstieg und Gesamtamplitudenabfall in der Beschleunigungs-Nystagmusperiode läßt auf eine mit dem Alter zunehmende Verschlechterung der Fähigkeit schließen, einen äußeren physikalischen Reiz in einen inneren phy iologischen zu transformieren.

SUMMARY

Tw earl er papers ga information on the age-depen lence f the tot l mpl tudes l frequen les l the time-limited nystagmu period. This paper report pecial examination carried out in the 1st perrotatori n stagmu period Th stagmus period oked by cceleration of test person is in led t acceleration nd a perrotat ric fading nyst gmus period Both th total mpl tudes d frequen les in these section of th 1st perrotat ri st gmus period as w ll the acceleration ystagmus period, preceding the latent period, were tested regardi g thei ge dependence The special aspect of the conduct f the total mplitudes nd frequen les during the cceleration nd the perrot t ric f d ng period as w ll a *the first-mentioned* y t gmus period, preceli g the latent period, for whi h s ignificant ge depe dence could be

proved in all cases, was shown The steeper age-dependent frequency increase and the decrease of the total amplitude during the acceleration nystagmus period —in contrast to the perrotatoric fading nystagmus period—leads to the conclusion that the capability to transform an outer physical stimulus into an inner physiological one decreases continually with age

LITERATUR

FLUUR, E., und MENDEL, L., 1966: R lation between strength of stimulus and duration of latency time in vestibular rotatory nystagmus. *Acta Otolaryng* (Stockh.) *61* 463.

FLUUR, E., MENDEL, L., und LAGERSTRÖM, L., 1966 Reproducibility of duration of latency tim in unidirectional per-rotatory nystagmus. *A ta Ot laryng* (Stockh.) *62* 118.

FRENZEL, H 1959 *Zur Frage einer Routinetechnik d r thermischen Vestibularerreg* *HNO* (Berl) 353

GROHMANN, R und MINNIGERODE, B., 1967: Gesamtamplitud und Frequenz als Be er *tungsgrößen des experimentell n Nystagmus. Acta Otolaryng* (Stockh.) *63* 331

MINNIGERODE, B und GROHMANN R 1966: Untersu hungen u Bestimmung der maßgebenden Charakt ristika de Elektronystagmogramms gesu der V rsuchspersonen verschied n n Lebensalters bei rotatorischen Beschleunigungsreizen *Acta Otol ryng* (Stockh) *61* 101

MINNIGERODE, B GROHMANN R und VOSTEN H 1967: El ktro ystagm graphische Untersuchungen zum Verhalten der Labyrinthfunktion gesunder V rsu hspersonen v r schieden n Lebensalter *Pract Otorhinolaryng* (Basel) *29* 153

MITTERMAIER, R 1939 Übe klinische Untersu hung n des Drehnach ystagmu *Arch Ohrenh Ilk 46* 305

DE NÓ LORENTE, R 1928 *Di Labyrinth efl x auf die Augenmuskeln* Urban & Schwar enberg, Wien.

SZENTÁGOTHAI, J 1952: *Die Roll de in In n Labyrinthr epi en bei der O ient ien von Augen und K pf im Raum.* Akadémiai kiado, Budapest.

Univ Hals-Nasen-Ohrenklinik Geiststr 5/10
34 Gött ng n Deutschl nd

Eingegangen am 15 De-ember 1966

Acta oto-laryngologica 61, 313-352 (1967)

DRUG CONTROL OF AROUSAL AND NYSTAGMIC HABITUATION IN THE CAT

J. H. Brown and J. E. Marshall
Fort Knox, Ky, U.S.A.

From the U.S. Army Medical Research Laboratory, Fort Knox

Nystagmic habituation was compared in 43 cat (1 per group) exposed to repeated angular acceleration following injection of d-amphetamine sulfat, Nembutal, or placebo. Although large differences in total slow phase nystagmic output were found between the groups, the habituation pattern across trials was essentially unaffected. The results indicate that nystagmic habituation in total darkness reflects more than a loss of arousal, and demonstrate the value of *d*-amphetamine sulfate as a useful experimental control in the study of nystagmic habituation.

Repeated exposure to vestibular stimulation leads to a marked reduction in the magnitude of the nystagmic response of pigeons (Mowrer 1934), cat (Crampton 1964) and humans (Collins, 1964). Although this diminished response has commonly been characterized as habituation, the extent to which these changes reflect a loss of mental alertness during testing rather than true habituation has not been ascertained. Wendt (1936, 1951) has suggested two processes to account for the response decrement. Like Dodge (1923) he argues that the decrement occurring when S has visual cues available during rotation reflects the resolution of competition between the functional systems effecting still fixation and the disruptive nystagmic response. However, Wendt departs from Dodge's suggestion that the decrement occurring when visual cues are not present results from a functional learning in which useless responses drop out. Instead, Wendt has argued that the decrement in darkness primarily reflects diminished mental alertness. This was based on general observations of the influence of excitement on nystagmus (Mowrer 1934 *b*) and more specifically inferred from Wendt's observation that decreases in nystagmic output of monkeys could be largely reversed by the presentation of alerting stimuli such as a banana odor (Wendt 1951).

Subsequent investigations have demonstrated the interaction of arousal level with the nystagmic response decrement (Collins, Crampton & Posner 1961; Collins, 1962; Collins & Poe 1962) but have not provided a critical test of Wendt's position. For example, Crampton & Schwam (1961) after observing that the habituated response of cats in darkness was accompanied by an EEG indicative of diminished arousal, found partial, temporary

recovery when alerting auditory stimuli were presented They also found a nystagmic decrement when cats were continuously alerted using cutaneous electric shock but pointed out the potential influence of sensory adaptation as limiting the alerting effects of the shock In a recent study examining the interaction of vision and arousal Marshall & Brown (1966) have found in humans, that *Ss habituated in darkness show a significant* slow phase increase when tested both with vision and an increased arousal level In contrast *Ss in a group required to perform a visual task during* the habituating trials showed a continued decline which was impervious to the effects of the increased arousal level

However none of the psychological tasks used in these studies permits the assumption that a continuously uniform level of mental alertness was maintained throughout relatively long experimental sessions In order to effect more uniform control of this important variable Crampton (1964) and Brown (1965) have used *d* amphetamine sulfate to maintain a high arousal level in cats, but not under conditions permitting a systematic comparison of the effects of drug-controlled arousal on the habituation process

The present study was designed primarily to compare nystagmic habituation under different conditions of drug-controlled arousal including *d-amphetamine sulfate Nembutal anesthesia and a placebo condition* Since surgical levels of anesthesia with Nembutal eliminate nystagmus, data from these *Ss* is also relevant to the problem of whether the response is necessary for nystagmic habituation or if exposure to the stimulus without occurrence of a response is sufficient

METHOD

Apparatus

Rotary

A circular horizontal turntable was driven about the vertical axis by means of a friction rim drive coupled to an electrohydraulic control system (Crampton & Brown 1961) Constant angular accelerations of precise duration and magnitude were programmed from control equipment outside the shielded ventilated lightproof room which housed the turntable

Restraint

Animals were restrained by the technique of Henriksson Fernandez & Kohut (1961) At least one week prior to testing *Ss* were anesthetized and small *holes drilled through the canine teeth* Prior to each experimental run S was carefully wrapped and placed in a special restraint box Piano wire (No 10) was strung through the drilled teeth inserted into a vise arrangement at either side and drawn taut The box was positioned on the turntable so that the head was bisected by the vertical axis of rotation

TABLE 1 *Experimental Design*

Group	Session 1 Trial 1	Session 2, Trials 1 10	Session 3, Trials 1 11
I	Amphetamine	Amphetamin	Amphetamine
II	Amphetamine	Nembutal	Amphetamine
III	Amphet min	Placebo	Amphetamine

Recording

Needle electrodes were inserted in folds of the kin at the outer canthi and a ground wire attached to the wire strung through the teeth of each *S* Electro-oculographic signals were led through slip rings to an ink writing recorder modified to permit recording with a 2.8-sec RC time constant

Procedure

The 45 *Ss* were mature cats who had not been previously exposed to constant angular acceleration Three groups of 15 cats each were tested on three separ te experimental sessions spaced seven days apart All groups recei ed identical acceleration experience with the independent variable consisting of the introduction of the different drug conditions on session 2 (Table 1) The cceleration program for each trial consisted of the follow ing (*a*) 1 sec of constant ; mill e acceleration (7 /sec²) (*b*) 80 sec of con tant v locity and (*c*) 500 sec of low level deceleration (0.25 /sec²)

On ses ion 1 ach S received a 2.5-mg kg intraperitoneal injection of d amphetamine sulf te After waiting 60–90 min to allow the *d*-ampheta min t tak effect th nimal was placed on the turnt ble and brought to 6 0 rpm with low-level acceleration The ro m wa illuminated to permit recording of optokinetic n stagmus as a calibration signal After obtaining the calibration th room lights w re turned off and S was returned to 0 0 rpm with a l w level deceleration After 10 min of total darkness, the first trial was begun Ses i on 1 con isted f only one tri l Nystagmu was monit red in t tal darknes on all trials At the end f each sessi n opto-k netic ny tagmu wa gain recorded for calibration

On ses i n 2 exactly the same procedure was followed, except that Group I w injected with *d*-amphet mine ulf t (2.5 mg kg) Group II with Nembut l (0 75 cc kg of 30 mg cc solution) nd Group III with a saline pl cebo (0 cc kg) All *Ss* were xposed to 10 trial during thi second session habituation series

I n th third ses i n (test series) all S gain received *d*-amphetamine ulf t 2.5 mg kg) and wer xposed to a series of 10 positive accelera tion trials The fin l trial f thi ses ion con i ted of a sec² negative gul r acceler tion t letermine if h bituati n to the 21 previ us positive celeration w uld dem n trate direction l transfer

recovery when alerting auditory stimuli were presented They also found a nystagmic decrement when cats were continuously alerted using cutaneous electric shock but pointed out the potential influence of sensory adaptation as limiting the alerting effects of the shock In a recent study examining the interaction of vision and arousal Marshall & Brown (1966) have found in humans, that *Ss* habituated in darkness show a significant slow phase increase when tested both with vision and an increased arousal level In contrast *Ss* in a group required to perform a visual task during the habituating trials showed a continued decline which was impervious to the effects of the increased arousal level

However none of the psychological tasks used in these studies permits the assumption that a continuously uniform level of mental alertness was maintained throughout relatively long experimental sessions In order to effect more uniform control of this important variable Crampton (1964) and Brown (1965) have used *d* amphetamine sulfate to maintain a high arousal level in cats, but not under conditions permitting a systematic comparison of the effects of drug-controlled arousal on the habituation process

The present study was designed primarily to compare nystagmic habituation under different conditions of drug-controlled arousal including *d*-amphetamine sulfate, Nembutal anesthesia and a placebo condition Since surgical levels of anesthesia with Nembutal eliminate nystagmus data from these *Ss* is also relevant to the problem of whether the response is necessary for nystagmic habituation or if exposure to the stimulus without occurrence of a response is sufficient

METHOD

Apparatus

Rotary

A circular horizontal turntable was driven about the vertical axis by means of a friction rim drive coupled to an electrohydraulic control system (Crampton & Brown 1964) Constant angular accelerations of precise duration and magnitude were programmed from control equipment outside the shielded ventilated lightproof room which housed the turntable

Restraint

Animals were restrained by the technique of Henriksson Fernandez & Kohut (1961) At least one week prior to testing *Ss* were anesthetized and small holes drilled through the canine teeth Prior to each experimental run *S* was carefully wrapped and placed in a special restraint box Piano wire (No 10) was strung through the drilled teeth inserted into a vise arrangement at either side and drawn taut The box was positioned on the turntable so that the head was bisected by the vertical axis of rotation

TABLE 1 *Experimental Design.*

Group	Session 1 Trial 1	Session 2, Trial 1 10	Session 3, Trials 1 11
I	Amphetamine	Amphetamine	Amphetamine
II	Amphetamine	Nembutal	Amphet mine
III	Amphetamine	Placebo	Amphetamine

Recording

Needle electrodes were inserted in folds of the skin at the outer canthi and a ground wire attached to the wire strung through the teeth of each S Electro-oculographic signals were led through slip rings to an ink writing recorder modified to permit recording with a 2.8-sec RC time constant

Procedure

The 45 Ss were mature cat who had not been previously exposed to constant angul r acceleration. Three groups of 15 cats each were tested on three separate experimental sessions spaced seven days apart All groups received identical acceleration experience with the independent variable con isting of the introduction of the different drug conditions on session 2 (T ble 1) The acceleration pr gram for each trial consisted of the follow in, (a) 15 sec of con tant positive acceleration (7 /sec²) (b) 80 sec of constant velocity and (c) 500 sec of low level deceleration (0.25 sec)

On session 1 each S received a 2.5-mg/kg intraperitoneal injection of d-amphetamine sulfate After waiting 60–90 min to llow the d-amphetamine to tak effect the animal was placed on the turntable and brought to 60 rpm with a low level acceleration. The room was illuminated to permit recording of opt kinetic nystagmu as a calibrati n signal After obtaining the calibr tion, the room lights were turned off and S was returned to 00 rpm with a low level decelerati n After 10 min of total darkness, the first trial was begun Ses ion 1 con isted f only one trial Ny tagmus wa monitored in total darknes on all trials. At the end of each session opto-k netic ny tagmu wa again recorded for calibration

On ses ion 2 exactly the sam procedure wa foll wed except that Group I wa injected with d amphetamine ulfate (2.5 mg kg) Group II with Nembutal (0 cc kg f 50 mg cc soluti n) and Group III with a saline placebo (0 cc kg) All Ss were exposed to 10 trials during thi secon l session (habituation series)

For the third ses ion (test series) all Ss again recei ed d-amphetamine ulf (2.5 mg kg) and were exposed to a series of 10 positive accelera tion trials. The fin l tri l of thi ses ion con i ted of a 7 sec negative angular acceleration t d termine if habituation to the 21 previou positi e acc lerations would demonstrat directi nal tran fer

The slow phase sweep of each primary nystagmic beat was measured first in mm for each 1 sec segment of the record summed across seconds for each trial and then converted to degrees of slow phase eye movement according to the calibration obtained at the end of each experimental session (Brown, 1965) These data were then treated logarithmically and geometric means computed by trials for each of the experimental groups.

RESULTS

In view of the extreme variability evident in nystagmic responses to angular acceleration logarithmic transformations and experimental designs amenable to analysis by covariance are necessary to adequately assess the effects of experimental manipulations The single trial on session 1 provided the covariate for data collected on sessions 2 and 3 Accordingly the data obtained on session 2 was subjected to analysis of covariance These data are plotted on the left of Fig 1 Since in no case was any output obtained from animals in Group II the Ss who were anesthetized were excluded from this analysis While both main effects were significant, Groups ($F = 5.13$ $df = 1/27$ $p < .05$) and Trials ($F = 13.84$ $df = 9/252$, $p < .01$) the Groups by Trials interaction was not significant ($F = 1.63$ $df = 9/252$ $p > .10$) This indicates that while the amphetamine treated animals have consistently higher output than the placebo animals both groups show statistically equivalent declines over trials. Since inspection of the data in Fig 1 indicated that the two groups might be starting to diverge at the end of 10 trials, additional animals were run in an extended second session The same procedures were followed as in the first experiment but with only five animals in each of the three groups and with session 2 consisting of 20 rather than 10 trials These data are plotted on the left side in Fig. 2 An identical analysis of covariance was carried out on these data with equivalent results i e both Groups and Trials were significant while the Groups X Trials interaction did not approach significance

Session 3 data for all three groups in experiment 1 also were analyzed by covariance using session 1 as the covariate While Trials again was significant ($F = 41.47$ $df = 9/378$ $p < .01$) neither Groups ($F = 0.13$ $df = 2/41$ $p > .25$) nor the Groups by Trials interaction ($F = 1.29$ $df = 18/378$, $p > .25$) approached significance These data are plotted on the right in Fig 1 Session 3 data for the 15 Ss in the second experiment are plotted on the right in Fig 2 Analysis of these data again yielded comparable results, a significant Trials main effect with Groups and the Groups X Trials interaction failing to approach significance

Finally the high level of output for all three groups on the final trial of session 3 should be noted This is clearly consistent with Crampton's (1964 finding that habituation obtained to positive angular acceleration does not transfer to negative angular acceleration

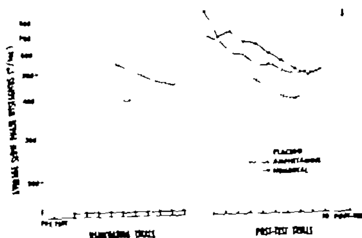


FIG. 1 A erage sl w phase nystagmu plotted log rithmically galnst trials (10 trial 1 ses ion 2)

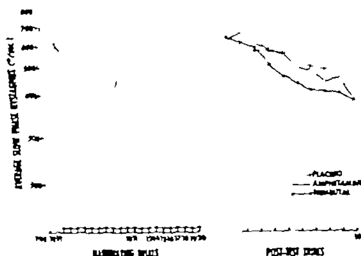


FIG. 2 A erag slow phase ny ag plotted loga ithmically galnst trial (20 trial) he session 2

DISCUSSION

There ppea to be three general conditions under which the horizontal component f the estibulo-ocular reflex will manifest a response decrement fir t that occurring primarily from a decrease in arousal (Wendt, 19 1) secon l decrement representing resolution of response compett t n between the vi ual and vestibular systems (Wendt, 19 1 Guedry 1961) third, the functional effect of learning in whi h useless (Dodge 1923 or n n-con ummatory (Thorpe 19) responses are eliminated. Examination f this third type of habituation ha e been complicated by

a failure to control for the first two types in that experiments typically have not included controls both for *S's* arousal level and his visual environment.

The significant group differences during the habituating sessions, together with the failure of the Groups × Trials interactions to approach significance indicate that while amphetamine leads to significantly more nystagmic output than is obtained from untreated animals, the slope of the habituation function is essentially unaffected by the amphetamine. This clearly indicates that nystagmic habituation does occur even under conditions of a continuously maintained high level of arousal. Since these data were obtained in complete darkness, it is also evident that competition between the visual and vestibular systems is not a prerequisite for habituation. As Crampton (1964) has pointed out, the habituated response is more closely tailored to the stimulus in that both the pre- and post-stimulus exuberance of the response are dramatically reduced. Although a similar tailoring is found in more complex experimental situations that permit competition between the visual and vestibular systems, the present results suggest that examination of habituation as a fundamental learning process is most meaningfully accomplished in darkness with careful control of arousal. That amphetamine increases the precision of experimental control by reducing the response variability resulting from spurious trial-to-trial changes in arousal level can be inferred from the differences in variability between the placebo ($\sigma_{Trial\ 3} = 124.3$, $\sigma_{Trial\ 6} = 119.$[illegible], $\sigma_{Trial\ 9} = 123.3$) and amphetamine ($\sigma_{Trial\ 3} = 107.1$, $\sigma_{Trial\ 6} = 106.4$, $\sigma_{Trial\ 9} = 105.0$) conditions.

The complete failure of the drug conditions on session 2 to influence session-3 data was unexpected. In both experiments all *Ss* had recovered to near pre-test levels during the week that separated sessions 2 and 3, precluding any sensitivity for the evaluation of potential differences between the three experimental groups. Previous findings have shown that there are no differences between massed and distributed experience for the acquisition of habituation, but retention is significantly affected in that massed experience leads to little if any retention (Brown, 1965). Since the present study provided massed experience, the nearly complete recovery of all groups would seem to be consistent. While this leaves unanswered the question of whether the actual occurrence of the response is critical to nystagmic habituation, these data are consistent with indications that exposure to the stimulus without occurrence of a response may be sufficient for habituation (Brown, 1966).

Recent evidence has indicated that amphetamine potentiates the efficacy of various anti-motion sickness agents (Wood, 1965), as well as being effective in and of itself in some cases (Vasilyev & Belay, 1964; Wood, 1965). These findings implicate the drug as potentially exerting a direct effect on the vestibular system. However, in view of the effects of the drug on the vestibulo-ocular reflex in the present study, it seems much more

likely that amphetamine affects the system only indirectly through some general changes in the physiological state of the organism That the effects of amphetamine on nystagmus appear to be identical to having *S* diligently perform mental arithmetic or some other attention demanding task (Collins & Poe 1962) buttresses this interpretation

RESUME

L'habitode nystagmiqu était comparée chez 45 chats (15 chats en ch que groupe) exposés à l'a célération angulaire répétée suivant l'injection de *d*-amphetami e sulfat Nembutal ou placebo. Bien que des gra des différences dan l'entière phase lente de produit nystagmique ont été rencontrées entre les groupes, l'habit de typique pendant les expériments était en général inaffectée Les résultats indiquent que l'habitude nystagmique dan l'obscurité complète réfléchit plus que la pert d l'irritation et même prouve la valeur d *d*-amphetamine sulf te comme un contrôle expérimental utile da l'étud d l'habitude nyst gmique

ZUSAMMENFASSUNG

Nyst gmische Gewöhnung wurde be 4 Katzen erglichen (j 15 in iner Gruppe) ausgesetzt zur wiederholten Winkelgeschwindigk it na h der Einsprit zung on *d* Amphetamin Sulf t, Nembutal oder Placebo Obgleich grössere Unterscheid in der ganzen langsamen Phase der ystagmischen Produktion fest gestellt wurden unter den Gruppen blieb d llgemeine Gewöhnungsmuster während der Experiment im ganzen unbeeinflusst. D e Ergebnisse zeigen dass die nystagmisch Gewöhnung i voller Dunkelheit mehr reflektiert al den Verlust on Erregung nd bezeugen den Wert on *d* Amphetami S lfat ls el e ötliche e periment le Überwachung in der U tersuchu g der ny tagmischen Gewöhnung.

REFERENCES

Lnow J H 1963 Acquisition nd retention of ystagmic habituation i cat with dist bated celeration perience *J Comp Phy iol Psy hol* *60* 310

1966 Interacting estibal stimuli d st gmic habituation. *Act Otol ryng* Stockh *62* 311

rns, W E., 1962 Effect f ment l act pon estibul ystagmus *J Exp. Psy hol* *63* 191

1961 T sk control of rousal nd the ffect of repeated nidirection l ngula acc ler ion on huma estibul responses *Act Otol ryng* (Stockh.) S ppl. 190 1

Col rns, W E., Cr wfor G. H nd Pos n, J D., 1961 Effect of ment l activity on est bul gmus and the EEG *N ture* 190 194

1962 Direction l imbal nce of estibula ystagm in cat f llowi g repeated idirectional gul acceler tion *Act Otolaryng* (Stockh.) 55, 41

Co rns, W E., nd Poe, R. H 1962 Amphet mi rousal, nd h ma estibular y tagm *J Pharmacol E p Ther* 138 120

Crampton, G H 1964: Habituation of ocular nystagmus of vestibular origin. In *The Oculomotor System* (ed. M B Bender) p 332 Harper & Row New York.

Crampton G H and Brown, J H 1964 Repeated vertical s micircular canal stimulation does n t habituate horizontal nystagmus in cat *Acta Otolaryng* (Stockh.) 58, 441

Crampton G H and Schwam, W 1961 Effects of arousal reaction on n stagmus habituation in the cat *Amer J Physiol* 200 29

Dodge, R., 1923 Habituation t rotation *J Exp Psychol* 6 1

Guedry F E., 1964: Psychophysiological studies of vestibular functi n. In *Contributions to Sensory Physiology* (ed W P Neff) Vol F A ad mic Press, New York.

Henriksson, N G Fernández, C., and Kohut R 1961: The caloric test in the cat. *Acta Otolaryng* (Stockh) 53 21

Marshall, J E., and Brown J H 1966 Visual arousal interaction nd pecificity of nystagmic habituation *USAMRL Rep* No 688 9 Dec 1966 Fort Knox, Kentucky

Mowrer, O H 1934 *a*: Th modification of vestibular nystagmus by mean of repeated elicitation *C mp. Psychol Monogr* 9 1

— 1934 *b* Influ nce of "excit ment on the duration of postrotational nystagmus. *Arch Otolaryng* (Chic) 19 46

Thorpe, W H 1956 *Learning and Instinct in Animals* Harvard Press, Cambridg

Vasilyev P V and Belay V Y 1964 Effect of some drug on toleratio to acceleration *NASA Tech. Translation F 228* 79

Wendt G R 1936 An interpretation f inhibition of conditioned reflexes a competiti n between reaction systems. *Psychol Rep* 43 258.

— 1951: Vestibul functions In *Handbook of Experimental Psychology* (ed. S. S. Stev ns) Wiley New Y rk

Wood, C. D 1965: Antim tion ickness drugs fo aerospace NASA SP 77 *The role of the vestibular organs in th explorati n of space* 365

U.S Army Medical Research Laboratory
Fort Knox Ky 40121 U.S.A

Received February 8 1967

Acta oto-laryngologica 61 333-388 (1967)

METASTASIS OF LARYNGEAL AND HYPO-PHARYNGEAL CARCINOMA

J. M. ALONSO
Montevideo Uruguay

The author has studied the mechanism which produces remote metastases in laryngeal and hypo-pharyngeal cancer and the line of approach by necropsy which is the most scientific, and by clinical observation, which is the most convenient in medical practice. The author's material has shown predominance in the male and where the primary lesion is of degree 2 or 3. It shows also the predominant localization of primary lesions and metastases, remote metastasis of cured carcinoma and the rare metastasis in the larynx and the hypopharynx of malignant tumours of other organs.

It is said that laryngeal carcinoma is a cancer "sur place" but even if it remains, in general, as an isolated factor or becomes metastasized to the regional lymph nodes, creating the regional-ganglionic lesion, it can also, in some instances, metastasize to some other regions of the body. I wish to offer some comment about these remote metastases, and to discuss the cases I have observed; there are some cases of multiple remote metastases of malignant tumours from other organs appearing in the larynx.

The remote metastases can be studied in the following manner:

(a) By pathological studies of autopsies of patients who have died from cancer of the larynx or have at one time suffered from it.

(b) By the clinical examination of patients who present or have presented laryngeal cancer and who show symptoms of having a tumour elsewhere.

(c) By the method known as "vital coloration".

It should be noted that the results reached by these three procedures cannot be superimposed.

The examination of material taken from autopsies offers more exact and scientific facts, because it allows comparative histopathological study both of the primitive tumour and that of the metastasis, but this refers to such cases as have reached the final stage and cannot serve as a guide in clinical practice.

The study of the vital coloration, applied to the lymphatic vessels, indicates the means of transfer of the lymph and the cells or neoplastic emboli therein carried, but the ganglionic metastases, for instance, modify the lymph flow.

The less scientific clinical facts are, however, most useful as a guide to the practitioner during treatment. Of course if all seriously-ill patients

were carefully studied and if after decease postmortem examinations were systematically carried out the number of proved cases of remote metastases would be greater

In this paper the study of the problem is based on the clinical facts, even though a study of the histo-pathology of the metastases is lacking in many of the cases which, therefore do not fit Russi s definition of the remote metastasis of a malignant tumour carcinoma i e "A metastasis is the transportation through the organism of pathologic products which proceed from any lesion and the remote reproduction of a lesion similar to that of the initial localization" This metastasis, as the primary lesion is capable of producing new metastases

It is traditional to say that epithelial type tumours spread by lymphatic means and that those of conjunctive type do so through the blood stream. We have seen that this old assertion has some elements of truth but it will be noted that the means of propagation of carcinomas tumours which constitute almost the totality of the malignant tumours observed in the larynx and hypo-pharynx, are in fact various

(*a*) Remote metastases from laryngeal cancer produced through lymphatic channels are rare in clinical experience they are particularly prevalent as axillar or mediastinal carcinomatous adenopathies.

Due to the valvular system of the lymphatic vessels the lymph penetrates by way of the marginal sinus, where the stream is slower There a few cells are stopped while others proceed to another lymph node or blood vessel Also coming by the blood stream they reach the lymph node by the nutritious artery When a lymphatic vessel is obstructed it enlarges and the lymphatic stream can become reversed

(*b*) Metastases by way of the blood are, in general produced via the veins which come from the tumour by erosion of the venous or arterial vessels invaded by the primitive tumour or by the regional adenopathies (the superior laryngeal artery the carotidean artery or its branches) by penetration of the lymph with cells or neoplastic emboli into the blood stream especially when the lymphatic circulation is disturbed by metastatic adenopathies

We know that the presence of carcinomatous cells in the blood of cancerous patients is insufficiant to produce remote metastases because arterial blood and the tissues themselves can arrest or annul their action This has been specifically studied by Hermann in connection with laryngeal carcinoma

So as in the lymphatic channels, the neoplastic cells encounter their first filter at the lymphatic regional nodes, the cells or emboli which enter the venous circulatory stream going to the heart and thence to the lung where they find the first and most important unavoidable filter

Several types of diffusion by the blood stream have been described but in laryngeal cancer this i produced generally through the venous system of the neck heart and lung (the unavoidable filter) If the cells pass this

filter entering into general circulation they can colonize in any part of the body

(*c*) The metastases known as preferential are observed in carcinomas of other organs (the thyroid and prostate glands in particular) but not in laryngeal tumours.

The same applies to the selective metastasization of Sching of tumours of the medulla, of citoid blastomas of the lymph nodes, etc.

(*d*) Another method of remote metastasis is by inoculation or graft. For the lung it may be admitted that this way of propagation may be assisted by manipulation of the tumour This process may be effected principally (1) for the bucal cavity and the esophagus in hypo-pharyngeal cancers, (2) for the bronchial tubes and lungs for laryngeal cancer If the larynx is moistened with a radio-opaque fluid the radiograph shows their pene tration into the bronchial tree.

(*e*) Difficult cases which give cause for thought are for instance, those where outside the tumour and separated from it by a zone of healthy mucous membrane or other tissues, there appear ulcers or infiltrations of neoplastic tissue as can be observed in the base of the tongue and the subglottis.

These cases make us believe that even when the histo-pathological study of the border of the extirpated tumour shows non-cancerous tissue, one cannot be sure of having obtained a total extirpation This is a reminder that in medicine especially in cancer nothing is absolute

(*f*) The cases of remote metastases of malignant tumours from other organs in the larynx are not common Thyroidean tumours are in fact, the spreading of primitive thyroidean tumours to the larynx (especially to the sub-glottis) and not remote metastases.

True metastases f this type seem to be cases of hypernephroma Tamura and Nakamoto and Köhle s cases.

(*g*) When cancer (laryngeal or other) has two simultaneous localiza tions it is sometimes very difficult to determin wh ther the two are independent tumours r one is metastatic from the other having been present for a long time without being noticed due to lack of symptoms or lack of observation

Remote metastases appear more frequently during the first two years after the surgical or physical treatment of the tumour Many times they appear when the primitive tumour i not cured but others appear when the original tumour i clinically ured.

It would seem that the surgical intervention, in particular when the tumour has been handled or incompletely extirpated, helps the appearence f remot metastases apparently it i the same with Roentgen or cobalt treatment

When the remote meta tases appear 10 years, or even later after the primitive cancer has been cured the cases (and these are not rare) are more difficult to interpret. I it a new carcinoma Is it a late metastasis

were carefully studied and if after decease postmortem examinations were systematically carried out the number of proved cases of remote metastases would be greater

In this paper the study of the problem is based on the clinical facts, even though a study of the histo-pathology of the metastases is lacking in many of the cases which, therefore do not fit Russi's definition of the remote metastasis of a malignant tumour carcinoma i e "A metastasis is the transportation through the organism of pathologic products which proceed from any lesion and the remote reproduction of a lesion similar to that of the initial localization This metastasis as the primary lesion is capable of producing new metastases

It is traditional to say that epithelial type tumours spread by lymphatic means and that those of conjunctive type do so through the blood stream. We have seen that this old assertion has some elements of truth but it will be noted that the means of propagation of carcinomas, tumours which constitute almost the totality of the malignant tumours observed in the larynx and hypo-pharynx are in fact various

(*a*) Remote metastases from laryngeal cancer produced through lymphatic channels are rare In clinical experience they are particularly prevalent as axillar or mediastinal carcinomatous adenopathies.

Due to the valvular system of the lymphatic vessels, the lymph penetrates by way of the marginal sinus where the stream is slower There a few cells are stopped while others proceed to another lymph node or blood vessel Also coming by the blood stream they reach the lymph node by the nutritious artery When a lymphatic vessel is obstructed it enlarges and the lymphatic stream can become reversed

(*b*) Metastases by way of the blood are in general produced via the veins which come from the tumour by erosion of the venous or arterial vessels invaded by the primitive tumour or by the regional adenopathies (the superior laryngeal artery the carotidean artery or its branches) by penetration of the lymph with cells or neoplastic emboli into the blood stream especially when the lymphatic circulation is disturbed by metastatic adenopathies.

We know that the presence of carcinomatous cells in the blood of cancerous patients is insufficiant to produce remote metastases, because arterial blood and the tissues themselves can arrest or annul their action This has been specifically studied by Hermann in connection with laryngeal carcinoma

So as in the lymphatic channels the neoplastic cells encounter their first filter at the lymphatic regional nodes, the cells or emboli which enter the venous circulatory stream going to the heart and thence to the lung where they find the first and most important unavoidable filter

Several types of diffusion by the blood stream have been described but in laryngeal cancer this is produced generally through the venous system of the neck heart and lung (the unavoidable filter) If the cells pass this

fter entering into general circulation they can colonize in any part of the body

(c) The metastases known as preferential are observed in carcinomas of other organs (the thyroid and prostate glands in particular) but not in laryngeal tumours.

The same applies to the selective metastasization of Sching, of tumours of the medulla, of ctioid blastomas of the lymph nodes, etc.

(d) Another method of remote metastasis is by inoculation or graft For the lung it may be admitted that this way of propagation may be assisted by manipulation of the tum ur This process may be effected principally (1) for the bucal cavity and the esophagus in hypo-pharyngeal cancers, (2) for the bronchial tubes and lungs for laryngeal cancer If the larynx is moistened with a radio-opaque fluid, the radiograph shows their pene tration into the bronchial tree.

(e) Difficult cases which give cause for thought are for instance, those where outside the tumour and separated from it by a zone of healthy mucous membrane or other tissues, there appear ulcers or infiltrations of neoplastic tissue as can be observed in the base of the tongue and the subglottis.

These cases make us believe that even when the histo-pathological study of the border of the extirpated tum ur shows non-cancerous tissue, one cannot be sure of having obtained a total extirpation This is a reminder that in medicine, especially in cancer nothing is absolute

(f) The cases of remote metastases of m lignant tumours from other organs in the larynx are not common Thyroidean tumours are, in fact, the spreading of primitive thyroidean tumours to the larynx (especially t the sub-glottis) and not remot metastases.

True metastases of this type seem to be cases of hypernephroma Ta mura and Nakamoto and Kohl s cases

(g) When cancer (laryngeal or other) has two simultaneous localiza tion it is sometimes very difficult to determine whether the two are independent tumours or one is metastatic from the other having been present for a long time with ut being noticed due to lack of ymptoms or lack of observation.

Remote m tastases appear more frequently during the first two years after the urgical or physical treatment of the tumour Many times they appear when th primitive tumour is not cured, but others appear when the original tumour is clinically cured

It would seem that the surgical intervention in particular when the tumour has been handled or incompletely xtirpated, helps the appearence of remote metastases pparently it is the same with Roentgen or cobalt treatment

When the remot metastases appear 10 years, or even later after the primitiv cancer has been cured, the cases (and these are not rare) are more difficult to interpret I it a new carcinoma Is it a late meta tasis

A palatine amygdala carcinoma—of the same histological type as a laryngeal tumour which has been extirpated 20 years before and has since been cured—is it a recurrence or a new tumour? (personal observation) An extirpated tumour of the left vocal cord cured A right carotidean adenopathy appears fifteen years later both are epidermoid carcinomas (personal observation) *I think this was a real remote metastasis.*

One can only be sure that they are two independent carcinomas *when one of them is an epidermoid carcinoma and the other is not.* When however they are tumours of the skin or of mucous membranes, frequently though not always (principally for tumours of the tonsil and base of the tongue) they are of the same histological type

PERSONAL STATISTICS

Many years work at the Maciel Hospital in Montevideo, and in private practice, permit me to offer statistics which I believe valuable even though most of the cases have no histological proof of their metastasis but only clinical and radiographic evidence.

Observations of the initial lesion are all perfectly recorded but very often the remote metastases have been treated at another surgery or hospital and autopsies are seldom performed

TABLE 1 *Remote metastases of laryngeal or hypo-pharyngeal cancer treated during 25 years*

1410 patie ts with cance f larynx o hypo-pharv x (dec ased) som after many y rs f treatm t fter cure f thei primary lesi

M l 1337 f m le 73	
Remote m t t ses	107
	(= 7.5%)
Simpl	88
Multipl	19
736 ryn ls, som f more th 20 y ars. 13 ll but with remot m ta t ses a t 31 t Dec mbe 1963 T t l, 5 7	

TABLE 2 *Grade or stage of initial lesion*

Grade	Cases
1	4
2	43
3	[illegible]
4	7

TABLE 3 *Primary lesion locali ation*

Supraglotti	
Epiglottic d estib l	52
V ntri le	11
Gl tti	26
Sub-gl tti	3
H po-ph ryn	15

TABLE 4 *Remote metastasis appearing less than 5 years after treatment of primitive tumour (in some cases the date of appearance of the metastasis was impossible to prove)*

Yea	
1	19
2	21
3	13
4	[illegible]

TABLE 5 *Remote metastasis appearing after 5 years*

Years	
At 5	9
From 6 to 10	14
From 11 to 15	8
At 16	2
At 20	1
At 23	3

TABLE 6 *Treatment of the primary lesion in the cases in which remote metastasis appeared*

Before 5 years	
S rgical	17
Radi tion	9
Both (SR or RS	1₀
After 5 y rs	
S rgical	17
Radiatio	1
SR or RS	19

TABLE 7 *Patients operated or treated, living as at 31st December 1965 736*

Male, 709; femal 27

Stage of primary lesio	
1	1
2	7
3	6
4	1

TABLE 8 *Remote metastasis with cure of primary lesion*

Year	Surgery	Radiation	RS	SR
1	0	0		3
3	0	[illegible]		1
4	0	0		1
6	[illegible]	0		1
7	1	[illegible]		0
11	0	1		[illegible]
12	0	0		1
14	[illegible]	[illegible]		1
15	1	1		0
17	1	0		[illegible]
Tot l	3	2		6

TABLES 9–11 *Localization of the metastasis*

Table 9 Digestive tract and annexes

Oral dy (Palatine nigdale, 3; tongue 5 gums, 1 Total, 9)	9
Hypo-phary	1
Esophagus	10
Stomach	3
Duodenum	1
Colon	1
Liver	3
Abdomen	3

Tabl 10 R piratory syst m and anne es

Cav m	3
Mediastine	5
Lungs (see Fig. 1)	30

Tabl 10 Urinary and genital system

Penis	1
Kidney	2
Urinary bladder	3

Tabl 10 b Several

Ea (pa illon)	1
Brains or endo-craneum	6

T bl 11 Bone syst m

Ocul orbit	2
Sk ll (see Fig 2	1
Pre-sternal or stern m	4
Shoulder	1
Thighs	3
Vertebral col mn	3
Sacrum	1
Hyoid bone	1

TABLE 12 *Multiple remote metastases*

Lips and esophagus	1
Palate and ear (pavillon)	1
Tongu and lungs	1
Tongue and mediastine	1
Mediastinal a d axilla nodes	1
Liver and pancreas	1
Skin of the chest and skin of other parts f the body (see Fig 3)	3
Vertebral column and lung	1
Multiple osseous	1
Multiple diverse	1

In Table 1 great predominance of laryngeal and hypo-pharyngeal cancer is observable in the male only 4.3% were female Single metastases were 4.5 times more frequent than multiple Later these are very rare in women

In Table 2 it is observed that generally the initial lesion was of grade 2 or 3

In Table 3 it is seen that in my patients the primary lesion was most frequent in the supraglottic localization

Most of the metastases in Table 4 appeared during the first two years after treatment or cure of the primary lesion

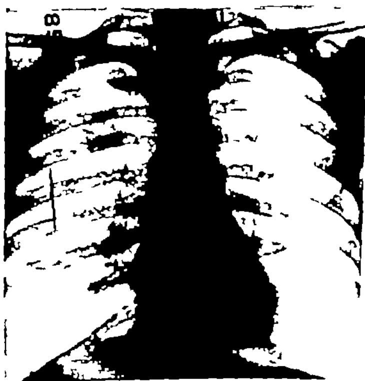


Fig 1 Lu g met t ses



FIG. 2. Skull metastases.

Table 5 is highly interesting It shows that remote metastasis may appear m ny years after th treatment and apparent cure of the primary lesion.

Table 6 shows that the class of treatment of the primary lesion does not seem to influence the frequency of appearence of metastases.

Table shows that amongst patients still living, the frequency of remote met tasis is higher when the primary lesion was of stage 2 or 3

In Table 8 we see that, when cure of the primary lesion has been obtained the h ice f surgical or radiotherapeutical treatment does not seem t be f ctor causing metastasis. Wh n both treatment have been used in combination, more cases of meta tases have been produced, but it should be observed that, generally speaking, such cases are more serious.

T bl 9 show that the most frequent meta tases of the digestiv tract are those f th esophagus. Those of the esophagu and of the oral cavity could, n many cases, be produced by the mechanism known as innoculat r grafting.

In Tal les 10 and 10 a we see that the most frequent metastases are those of the lung in the respiratory system and of the bladder in the urinary system In the craneal cavity they are seen with some frequency



FIG 3. Skin m tast ses.

In metastases of the bone those of the sternum vertebral column and thighs seem to be the most common

Laryngeal metastases of primitive malignant tumours in other regions are exceptional I nave never seen them but as mentioned previously there are reported cases of hyper nephroma

RESUMÉ

L auteur étudie la mécanique de production des metastases eloignées des carcinomes du larynx et du hipo-pharynx et la façon de faire leur étude par la nécropsie la plus scientifique par la clinique la plus convenable à la pratique médicale Le matériel de l auteur revèle prédominance chez l'homme ainsi que lorsque la lésion primitive était de dégré 2 u 3 On montre la localisation prédominante des lésions primaires ainsi que de métastases, les m tastases éloignées des carcinomes guéris et les très rares métastases les carcinomes ou tumeurs malignes l autres organes lans le larynx ou l hipo-pharynx

M reed 1
M ale deo l ruga f

R ceived F bru g 1 196

Acta oto-laryngologica 64, 363–372 (1967)

TRANSCONIOSCOPY IN THE STUDY OF THE MUCUS FLOW IN THE HUMAN LARYNX

B. Mårtensson and G. Ewert
Stockholm, Sweden

From the Department of Otolaryngology (Head: Prof. C. A. Hamberger) Karolinska Sjukhuset, Stockholm

Transconioscopy has made it possible for the first time to study the mucus flow in the human larynx. Such studies were made in 30 patients. It was found that, over normal laryngeal mucosa, the mucus from the trachea is, as a rule, transported to the posterior commissure. The mucus from the anterior and lateral walls of the trachea then deviates in a dorsally directed flow on the under-surfaces of the vocal cords, and runs parallel to the edges of the cords toward the posterior commissure. In a few cases, a flow pattern was observed in which all mucus was transported to the anterior commissure. In all cases hitherto verified histologically, areas with inhibited mucus flow have proved to correspond to areas of metaplastic epithelium not detectable macroscopically. In cases of laryngeal carcinoma, such areas consistently surrounded cancerous tissue. This metaplastic epithelium was also observed to cover areas in which the tumour grew submucously. It is pointed out that studies of the mucus flow by means of transconioscopy may be of great clinical importance for determining the extent of malignant laryngeal disease.

Studies of the transport of mucus in the larynx have previously been made only *in vitro* in animals. No *in vivo* studies have been reported, either in animals or in man. This is presumably to be ascribed partly to the difficulty of inspecting the laryngeal mucosa under physiological conditions. Another reason is that the most interesting part of the larynx from the point of view of the transport of mucus—*i.e.* the subglottic space—is hard to inspect with conventional methods of examination.

The introduction of a new method of inspection—transconioscopy—has, however, made it possible to follow under relatively physiological conditions, the transport of mucus from the trachea, through the subglottic space over the plane of the vocal cords.

EPITHELIAL DISTRIBUTION AND MUCUS FLOW PATTERNS

One of the prerequisites for the transport of mucus is the existence of a ciliated cylindrical epithelium. The epithelial distribution in the larynx has been described by many authors, among them Hopp (1955), Hilding

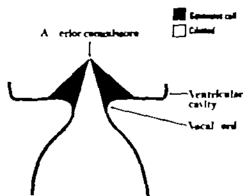




Fig 1

Fig 2

Fig. 1 Distributi n of squamous epithelium n the vocal cords. Squamo pitheli m co ers th fre edg f the cord, and forms a sharp borderli e ag in t the ciliated epi th lium in the ventricular cavity and on the under surface of th cord. Ciliated epithelium may be pre ent in the terio commis ure

Fig. 2. Instrum nts f r transconiosc py

(1956) and Jelinek (1966) The subglottic space, together with the greater part of the under surface of the vocal cords, is lined with ciliated cylindrical epithelium Squamous-cell metaplasia is presumably somewhat rare at this site Thus, Jelinek found subglottic metaplasia in only 1 of 67 macroscopically normal laryngeal specimens The cylindrical epithelium on the under surface of the vocal cords forms a sharp borderline against the squamous epithelium lining the free edge of the cords. This zone of squamous epithelium is fairly narrow and its extent in the cranio-caudal direction ranges from 1 2 to 2.5 mm on the under surface of the cord and from 0 8 to 2.5 mm on the upper surface (Jelinek 1966) On the upper surface the squamous epithelium once more forms a sharp borderline against the cylindrical epithelium in the ventricular cavity (Fig 1)

Hilding (1956) described the presence of a narrow zone of ciliated cylindrical epithelium in the anterior commissure as well as of strands of this epithelium in the bottom of the vertical folds of mucosa in the posterior commissure This implies that a transport of mucus from the sub- to the supraglottic region could take place in these two areas.

Hilding (1956 1959) found the following pattern of mucus flow in *in vitro* studies on the calf larynx The mucus on the posterior wall of the trachea is transported in the directly cranial direction through the posterior commissure The mucus on the anterior and lateral walls flows in a directly

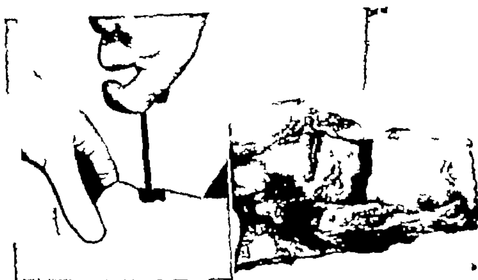


Fig. 1. Puncture of the cricothyroid membrane. The trocar penetrates the larynx about 1 cm caudal to the edge of the vocal cord ( ). Here the posterior wall consists of the strong cricoid lamina, which prevents damage to the oesophagus (b).

cranial direction to the level of the vocal cords. Here the flow deviates dorsally in a more or less even arch, and on the under surface of the cord runs parallel with the edge of the cord towards the posterior commissure where it once again deviates in the cranial direction. Thus, all mucus was transported to the posterior commissure and no flow was observed through the anterior commissure or over the edge of the vocal cords.

The present study was made to ascertain whether an equally regular pattern of mucus flow exists in the human larynx, and whether disturbances in the pattern can be correlated to metaplasia of the mucosa that cannot be observed macroscopically.

METHODS

The transport of mucus was studied by transconioscopy, inspection being performed through an angle telescope introduced percutaneously into the subglottic space. This method of inspection was devised and tested clinically at Karolinska Sjukhuset, Stockholm (Mårtensson *et al.*, 1964; Mårtensson, 1967 *a*, *b*). It is now used routinely for determining the extent of various diseases of the larynx, and has hitherto been carried out more than 200 times without any complications.

In our studies of the mucus flow, some minor modifications were made in the technique.

Premedication and anaesthesia

No premedication was given, since the drugs generally used—morphine, scopolamine and atropine—decrease the production of mucus in the airways, and most often completely inhibit its transport. The skin over the



FIG. 4 Positi n of the angle telescope in the subglottic space

cricothyroid membrane is anaesthetized with 2% Xylocaine exadrine* The membrane is then punctured with a fine needle and about 1 ml of 4% Xylocaine is applied as surface anaesthesia in the subglottic space In a study of the effect of local anaesthetics on the transport of mucus in the nose in man and in the trachea of the cat one of us found that 4% Xylocaine—in contrast to 2% Tetracaine—does not affect this transport (Ewert 1967) This has also been found to apply to the parts of the larynx studied

Puncture

The patient is placed in the tracheotomy position and a horizontal incision $^1/_2$–1 cm long is made in the skin over the cricothyroid membrane A trocar with an outer casing 4 mm in diameter is bored through this incision and the membrane punctured (Figs 2 and 3)

Passage of the outer casing through the membrane is observed at once by an instantaneous cessation of resistance The trocar is removed, whereas the outer casing is kept in place in the subglottic space and a right angled telescope provided with fibre illumination is introduced through it (Fig 4) Fibre illumination is essential in this connexion since it gives a "cold light" which implies that the mucosa is not exposed to the effect of heat during inspection

Inspection

The telescope has a wide range of mobility in the subglottic space It can be set at an angle upwards to the level of the rima glottidis, and downwards several centimetres into the trachea Concurrent rotation in the longitudinal axis of the instrument allows inspection of the edges of the vocal cords, the anterior and posterior commissures, the upper part of the trachea, and the whole subglottic space with the exception of a small area around the actual site of puncture (Fig 5)

The picture of the mucosa seen through the telescope is magnified, the degree of magnification being in inverse relation to the distance between the telescope and the area inspected. In view of this magnification, the direction of flow can, as a rule easily be determined by following the small air bubbles which are generally present in large quantities in the mucus. In some cases, we also used a tracer powder (Dibasic calcium phosphate 97 and Edicol Supra Orange 3%) It was then seen that the air bubbles in the mucus and the grains of tracer powder on its surface followed the same paths, and had the same rate

The rate of flow could not be determined exactly but was only estimated This was due to the fact that the varying degree of magnification makes it impossible to measure exactly the distance covered by the flow of mucus during a given time.

PRACTICAL APPLICATION

Studies of the mucus flow were made in 30 cases. In 6 of them, no macroscopically visible pathological changes were present in the larynx These patients were hoarse as a result of so-called phonasthenia, and transconioscopy was performed to rule out the existence of a subglottic neoplasm. From the point of view of the mucosa, they can be denoted as normal cases.

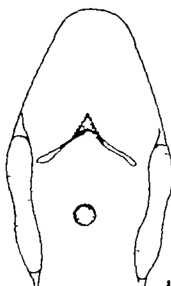
The diagnosis in the other 24 cases was as follows chronic laryngitis (7 cases) papilloma of the vocal cord (1 case) carcinoma of the hypopharynx (1 case) supraglottic carcinoma of the larynx (3 cases) and carcinoma of th vocal cord (12 cases)

In 7 of these 24 cases, the mucosa of the subglottic space was macroscopically normal In 10 cases, unilateral changes were present in the epi thelium f th ubglottic space here the transport of mucus over macroscopically normal mucosa took place in the healthy side of the subglottic space Th patient with a papill ma of the vocal cord had macroscopically l ible lesion f the mucosa in both sides of the ubglottic pace. The flow of muc o er macroscopically n rmal parts of the larynx could thus be studied in 20 of 30 cases.

Biopsy pecimens of the laryngeal mucosa were examined, and in some c ses the epithelial distribution could be accurately determined after cord- ct my nd laryngectomy

Tran port of Mucus ove Macroscopically Normal Parts of the Larynx

I most cases, we f und that the muc was transported to the posterior commi re in the same way as that previously observed in animals. We ha e denoted thi flow as the normal flow pattern In some cases, we f und a reversed flow all mucus being transported to the anterior commi ure Finally no transport of muc could be bserved in certain cases.



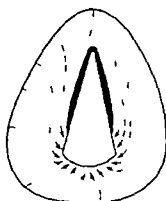


Fig 5 Fig 6

Fig 5 Parts of the larynx in pectabl at transcoloscopy (whit are)
Fig. 6 Th rima glottidis inspected from below at transconi scopy Arrow indicat the normal direction of mucus fl w towards the posterio commis ure Bl ck indicates the a ea without mucus flow

Normal flow pattern

In 19 of 29 cases, we observed a rapid flow of mucus which followed the pattern described by Hilding in animals All mucus was transported to the posterior commissure where it stagnated The mucus from the anterior and lateral walls of the trachea deviated in the dorsal direction on the under surface of the vocal cord and was transported parallel to the edge of the cord towards the posterior commissure This dorsally directed flow was observed up to about 2 mm from the edge of the vocal cords Cranial to this limit, no flow was visible (Fig 6)

This flow pattern was seen in 4 of the 6 normal cases, and in 15 of the 23 with laryngeal disease

The rate of flow was estimated at 5–15 mm/min which is in good agreement with the finding in the trachea of the rat (Dalhamn 1956) as well as with that in the human nasal mucosa (Ewert 1965)

Reversed flow

A reversed flow was observed in 3 cases. All mucus on the under surfaces of the vocal cords was transported in the ventral direction to the anterior commissure The flow over the under surfaces of the cords took place up to a few millimetres from their edge The rate was evaluated as normal

Since 2 of the cases were normal ones, no biopsy specimen was taken In the third patient, who had a localized carcinoma of one vocal cord histological examination showed the presence of cylindrical epithelium in the anterior commissure

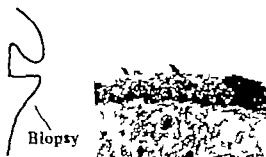


Fig. 7 Metaplastic, several-layered squamous epithelium from th under-surface f the ocal cord.

Inhibited flow

In 7 cases, no transport of mucus could be observed over macroscopically normal mucosa.

In 5 of these cases, the mucus was stagnant in both the trachea and the subglottic space In 2 of them Tetracaine anaesthesia had been given in connexion with ther examinations 1 and 4 days, respectively before transconioscopy The other 3 patients had chronic laryngo-tracheitis, with an xceedingly viscous mucus. The inhibited transport of mucus in these cases can, therefore, be explained by other factors than changes in the structure of the mucosa, i.e. the effect of local anaesthesia and high viscosity f the mucus.

In the 2 remaining cases, on the ther hand, we found normal transport over the tra hea and the vertical walls of the subglottic space but completely tagnant mucus on the under-surfaces of the vocal cords. In both cases, hist logical examination showed that the cylindrical epithelium on the under surfaces f the cord lacked cilia, or was replaced by squamous epith lium (Fig 7)

Transpo t f Mucus Around Carcinoma of the Vocal Cord

I all 12 cases with unilateral carcinoma of the vocal co d, the tumour wa f und t be surrounded by a zon of macroscopically normal mucosa, in whi h n flow of mucus occurred (Fig. 8) The ize of this zone ranged from few m ll m tres to over 1 cm

In 7 cases treatment had been confined to irradiation, so that no material was ailabl f r hi tological examination of the epithelium In the other cases, cordectomy r laryngectomy had been performed Histological examination showed n area of metaplastic epithelium, lacking cilia Its extent corresponded to the zone in which inhibited transport of mucus had been bserved at transconioscopy

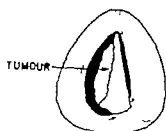


Fig 8

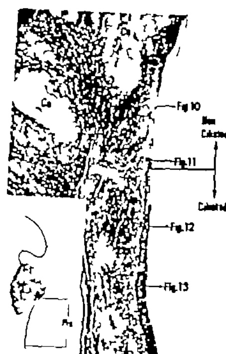


Fig 9

Fig. 8 Typical asymmetric mucus flow in a case of unilateral carcinoma of the vocal cord *The tumour is surrounded by an area of macroscopically normal epithelium without mucus flow (black area)*

Fig. 9 Survey of the mucosa caudal to a carcinoma of the vocal cord with subglottic growth *The thick arrow indicates the borderline between ciliated and non-ciliated epithelium, i.e. the caudal limit of the area without mucus flow Thin arrows mark the position of the detailed Figs. 10–13*

The borderline between ciliated and metaplastic epithelium was distinct, as illustrated in Fig 9 In this case the tumour involved the whole vocal cord and the cranial parts of the subglottic space *It also infiltrated submucously* in the caudal direction

A broad zone of metaplastic epithelium was present caudal to the tumour growing on the surface Directly *caudal* to the tumour there was definite squamous differentiation of the epithelium Continuing in the caudal direction this passed into a highly atypical non-ciliated cylindrical epithelium which with a distinct borderline was succeeded by slightly atypical but ciliated epithelium This borderline comprised the limit of the zone without transport of mucus (Figs. 9–13)

It can also be seen that the tumour grew submucously below the whole area of non-ciliated epithelium i.e. the area in which no flow of mucus was observed (Figs. 9 and 11)

DISCUSSION

Transconioscopy has proved to be a suitable method for studying the transport of mucus in the larynx The entire subglottic space can be inspected and due to the magnification in the telescope the flow of mucus





Fig. 10. Metaplastic squamous-cell epithelium slightly [illegible] the [illegible] on the surface (see Fig. 9).

Fig. 11. Highly atypical cylindrical epithelium, lacking cilia, covers the [illegible] growing tumour (see Fig. 9).

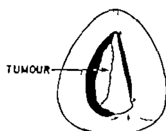


Fig 8

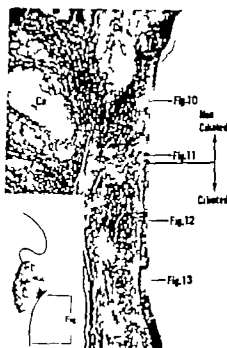


Fig 9

Fig. 8 Typical asymmetric mucus flow in a case of unilateral carcinoma of the vocal cord The tumour is surrounded by an area of macroscopically normal epithelium without mucus flow (black area)

Fig. 9 Survey of the mucosa caudal to a carcinoma of the vocal cord with subglottic growth The thick arrow indicates the borderline between ciliated and non-ciliated epithelium, i.e the caudal limit of the area without mucus flow Thin arrows mark the position of the detailed Figs. 10–13

The borderline between ciliated and metaplastic epithelium was distinct, as illustrated in Fig 9 In this case the tumour involved the whole vocal cord and the cranial parts of the subglottic space It also infiltrated submucously in the caudal direction

A broad zone of metaplastic epithelium was present caudal to the tumour growing on the surface Directly caudal to the tumour there was definite squamous differentiation of the epithelium Continuing in the caudal direction this passed into a highly atypical non-ciliated cylindrical epithelium which with a distinct borderline was succeeded by slightly atypical but ciliated epithelium This borderline comprised the limit of the zone without transport of mucus (Figs 9–13)

It can also be seen that the tumour grew submucously below the whole area of non-ciliated epithelium i.e., the area in which no flow of mucus was observed (Figs 9 and 11)

DISCUSSION

Transconioscopy has proved to be a suitable method for studying the transport of mucus in the larynx The entire subglottic space can be inspected and due to the magnification in the telescope the flow of mucus

without difficulty. In most cases, we found a distinctly mucus over macroscopically normal parts of the larynx, lish its pattern.

le to show that this pattern is most often identical to that *vitro* studies in animals, all mucus being transported to the missure. However in a few cases, the flow was reversed, and s transported to the anterior commissure.

il value of the method lies in the fact that inspection of the provides information about the state of the mucosa. We found ith macroscopically normal mucosa, but inhibited mucus flow zones with epithelial metaplasia, which were consistently seen cerous tissue.

wever essential to point out that the transport of mucus is not only on the presence of ciliated cylindrical epithelium. Naturally lity and iscosity of the mucus are highly important factors. ore, the mucus can well be envisaged to stagnate caudal to a on purely mechanical grounds, without any change in the epi- in thi area. The definite statement that can be made on the basis sconioscopy is that, in those areas in which mucus flows at an nished rate the mucosa is composed of ciliated cylindrical epi- n.

ording to Kleinsasser (1963) a laryngeal carcinoma is, as a rule, unded by a zone of metaplastic epithelium, often of precancerous type. r cases which could be verified histologically we found that the area hich the mucus flow was inhibited did in fact, correspond to the area met plastic epithelium. A highly interesting observation is that this helium may represent the area of submucous inv lvement by the tu or. Thus, our experience hitherto indicates that studies of the mucus w by means of transconioscopy may be of great importance for determin g the extent of a laryngeal carcin ma. They may perhaps, even permit the tent of ubmucous in olvement to be established.

ZUSAMMENFASSUNG

Die Transkonioskop hat es zum ersten Male mögl h gemacht, den Schleimfluss im menschlichen Kehlkopf zu st dieren. D ese Studien wurden n 30 Patienten durchgeführt. Man fan l, dass auf der ormalen laryngealen Schleimhaut in der Regel der Schle m on der Luftröhre zur h nteren K mmissur tran portiert wird. Der Schle m der hinteren und se lll hen W nd der Luftröhre w icht in em rückw rt gen Fluss uf der unteren Fläche der Stimmb d der b und fliesst pa allel zu den Kanten der Stimmbänder zur hinteren Kommissu. In einigen w igen F llen w de n Fliessmuster beobachtet, wobei aller Schleim zur vor deren Kommissur t sportiert wurde.

I allen b sher hist logisch untersuchten F llen hat es si h gezeigt, dass Gebiet w der Schleimfluss aufgehoben ist, den Gebieten mit Metaplasien des





FIG. 12. Furthe dal in the ubgl ttic pace there l lightl t ple l b t illated cyl drle l p th l m see Fig 9)

FIG. 13 Con l t tl llated c llndri l epith llum l prese t further c dall l th bgl ttl spa see F g 9)

be ae·ved without difficulty In most cases, we found a distinctly visible flow of mucus over macroscopically normal parts of the larynx and could establish its pattern.

We were able to show that this pattern is most often identical to that observed in *in vitro* studies in animals, all mucus being transported to the posterior commissure However in a few cases, the flow was reversed and all mucus was transported to the anterior commissure.

The clinical value of the method lies in the fact that inspection of the mucus flow provides information about the state of the mucosa. We found that areas with macroscopically normal mucosa, but inhibited mucus flow consisted of zones with epithelial metaplasia, which were consistently seen around cancerous tissue.

It is, however essential to point out that the transport of mucus is not dependent only on the presence of ciliated cylindrical epithelium Naturally the quality and viscosity of the mucus are highly important factors. Furthermore, the mucus can well be envisaged to stagnate caudal to a tumour on purely mechanical grounds, without any change in the epi thelium in this area. The definite statement that can be made on the basis of transconioscopy is that in those areas in which mucus flows at an undiminished rate the mucosa is composed of ciliated cylindrical epi thelium.

According to Kleinsasser (1963) a laryngeal carcinoma is, as a rule, surrounded by a zone of metaplastic epithelium, often of precancerous type. In our cases which could be verified histologically we found that the area in which the mucu flow was inhibited did, in fact, correspond to the area with metaplastic epithelium A highly interesting observation is that this epithelium may represent the area of submucous in ol ement by the tumour Thus, our experience hitherto indicates that studies of the mucus flow by means of transconioscopy may be of great importance for determin ing the extent of a laryngeal carcinoma They may perhaps even permit the extent of submucous involvement to be established

ZUSAMMENFASSUNG

Die Transkonioskopie hat es zum ersten M le mögl h gem ht, den Schleimfluss im menschlichen Kehlkopf zu studiere Diese Studi n wurden 30 Pat en t durchgeführt. Man fand da auf der n rmal n lary gealen Schle mhaut der Regel der Schleim von der Luftröhre zur hinteren Komm sur transportiert ird. Der Schleim d hl tere und seitl hen Wände der Luftröhre w icht in einem rückw rtigen Flus auf der leren Fla he der Stimmbä der ab und fliesst, parallel zu den Kanten der Stimmbä der zur hint ren K mmissur I l igen einigen Fällen wurd m Flussmuster beobachtet, wobei ller Schl im zu vor deren Kommissur transportiert wurde

I llen bi her histologisch n tersuchten Fällen hat es sich gezeigt, d Gebiete w der Schleimfluss gehobe ist, den Gebieten mit Metapla ien des

Epithels, die makroskopisch nicht zu beobachten sind entsprechen. In Fällen mit Kehlkopfkarzinom umgaben solche Gebiete immer das Karzinomgewebe. Dieses *metaplastische Epithel* wurde auch in Gebieten beobachtet, in welchen der Tumor submukös wuchs. Es wird betont dass Studien des Schleimflusses durch Transkonioskopie von grosser klinischer Wichtigkeit sein können zur Bestimmung *der Ausdehnung von malignen laryngealen Krankheiten*

REFERENCES

Dalhamn T 1956 Mucous flow and ciliary activity in the trachea of healthy rat and rat exposed to respiratory irritant gases *Acta Physiol Scand* 36 Suppl 1.3.

Ewert G 1965 On the mucu flow rate in the human nose *Acta Otolaryng* Suppl. 200.

— 1967 The effect of two t pical anaesthetic drugs on the mucus flow in the respiratory tract *Ann Otol* 6 359

Hilding, A C., 1956: On cigarette m king bronchial carcin ma and ciliary action. II Ciliary stre ming through the larynx and distribution of laryngeal epithelium *Ann Otol* 65 736

— 1959 *Ciliary streaming through th larynx and trachea* R lation t directio of ciliary beat and significance in sites of respiratory disease *J Thor Cardiov Surg* 37 108

Hopp E. S 1955 The devel pme t of th epith lium of the larynx. *Laryngoscope* 65 475

Jelinek, R 1966: Di Ausbreitu g des Platten u d Flimmerepithels im K hlkopf *Z Laryng Rhinol Ot l* 45 1

Kleinsasser, O 1963 *Cber di Hist g nese und das Wachstum f g r K hlkopfkrebse* *Z Laryng Rhinol Ot l* 42 490

Mårtensso B 1967 *a* Tr nsconi scopy A method fo improvement f end laryng al diag osis. *P act Otorhinolaryng* (Basel) 29 217

— 1967 *b*: Transc scopy in topical di gnosis f carcin ma of th l rynx. *Pract Oto-rhin laryng* (Basel) 29 233

Mårtensson B Fluur, E., nd Schiratzki, H 1964 Transconi scopy A new method of l rynge l investig ti *A ta Ot laryng (Stockh)* 58 281

Dept f Otolaryngology
Karolinska Sjukhuset
Stockholm 60 Stockholm

Rece ved March 31 1967

Acta oto-laryngologica 64, 373–387 (1967)

DURATION OF ANGULAR ACCELERATION AND OCULAR NYSTAGMUS FROM CAT AND MAN

I Responses from the Lateral and the Vertical Canals to Two Stimulus Durations

W. E. Collins and F. E. Guedry Jr.
Oklahoma City Okla. and Pensacola Fla U.S.A

From the Psychology Laboratory Civil Aeromedical Institute FAA Oklahoma City and the U.S. Naval Aerospace Medical Institute Pensacola

Recordings of ocular nystagmus were obtained from a group of cats and a group of human subjects to 4°/sec² angular acceleration of 8.4 sec and of 36 sec duration. Lateral canals and vertical canals were stimulated on separate trials. Results showed that the output of both primary and secondary nystagmus was greater for lateral canals. In cats, both lateral- and vertical-canal responses to the 36 sec stimuli peaked after 15–21 sec of angular acceleration and this was followed by a steady decline. Declines were not apparent in nystagmus of human subjects. A further test of these findings was conducted by manipulating arousal variables: human subjects were given special tasks and cats received d-amphetamine. Essentially the same results were obtained as described above. Other differences between the two groups were noted. Cats consistently demonstrated secondary nystagmus whereas humans did not. After termination of acceleration primary nystagmus from cats lasted longer and exhibited a greater number of eye movements following the 8.4 sec stimulus than following the 36 sec stimulus; this consistency was not evident in humans. However, for humans the sensation of motion following termination of acceleration was of longer duration for the 8.4 sec stimulus than for the 36 sec stimulus. In this regard nystagmus from cats resembled the subjective reactions of man more than they did the nystagmus of man.

INTRODUCTION

During prolonged angular acceleration inertial torque deflects the cupula but the deflection is eventually balanced, primarily by the cupula's elastic restorative force. Assuming that cupula displacement from its position of the equilibrium controls the magnitude of vestibular reactions, a prolonged angular acceleration should, according to the "Torsion Pendulum" theory (Groen, 1960) yield an increasing response for about 20–30 sec

this response level should be maintained without decline as long as the angular acceleration continues Several authors (Dodge 1923 Mittermaier & Rossberg, 1956 Montandon & Fumeaux, 1957 Guedry & Ceran, 1959 Ek, Jongkees & Klijn 1960) have reported that the subjective velocity rises and declines during prolonged angular acceleration contrary to theoretical expectations. Guedry & Ceran (1959) showed that the temporal period required for the subjective reaction to peak (and then decline) was about constant (27 sec) for angular accelerations ranging in magnitude from 0.5 to 2 /sec² Subsequent experiments (Guedry & Cunat unpublished) showed this to be true for stimuli up to 4 /sec²

The present experiments seek to compare cat and man in regard to several characteristics of the nystagmic response elicited by two durations of a 4 /sec² angular acceleration

METHOD

Cats

Apparatus

The Huffman Rotation Device (Collins & Huffman 1964) located in a light proof room, was used to produce acceleration programs. Animals were tested on the rotator in pairs with their heads at the center of rotation One cat box was secured to runners on the turntable and the second box was secured to a framed tier arrangement above the first box (Collins & Updegraff 1966)

Restraint

Cats were restrained by the method of Henriksson Fernández & Kohut (1961) Three or more days prior to testing the animals were anesthetized and holes were drilled transversely through their canine teeth At the same time fur around the ocular orbits was shaved off and a guideline for positioning the head was drawn with washable ink from the canthus to the tragus on each side For testing each animal was wrapped in a towel and placed in a cat box A strand of piano wire was inserted through the holes in the canine teeth The wire was held securely and the head of the animal was positioned by means of an adjustable device on the front of the box

Recording

For recording horizontal components of eye movements, needle electrodes were inserted by the outer canthi Vertical components were obtained by means of surface electrodes taped above and below the left eye The recorder was an Offner Type R Dynograph with 3-sec time constants used in amplification Prior to testing, animals were placed in an optokinetic stimulator a drum speed of 24 sec was used to obtain data for calibration purposes.

TABLE 1 *Order of stimulus presentation*

All trials comprised stimuli of 4 /sec² Duration of the stimulus was either 8.4 or 36 seconds L and V refer respectively to lateral and vertical canal stimulation.

Human subjects	Cats	Rotation direction	Trials 1	2	3	4
Or & W	100 & 101	CW	8.4 L	36.0 L	8.4 V	36.0 V
N & Jo	102 & 103	CCW	36.0 L	8.4 L	36.0 V	8.4 V
Pr & M	104 & 105	CW	8.4 V	36.0 V	8.4 L	36.0 L
F & Ma	106 & 107	CCW	36.0 V	8.4 V	36.0 L	8.4 L

Human Subjects

Apparatus

A Stille Werner RS-3 rotating chair situated in a light proof room, provided the acceleratory stimuli for the human subjects.

Recording

A pair of surface electrodes, taped by the outer canthi of the eyes, detected horizontal eye movements, while a second pair was positioned above and below the left eye for the recording of vertical eye movements. An Offner Type T polygraph with a 3-sec time constant was used in amplifying and recording the eye movement signals. Eye calibrations were obtained prior to each test by means of a calibration chart located on one wall of the rotation room.

Procedure

Each of eight cats and eight human subjects received 2 angular accelerations (for 8.4 and 36 sec) stimulating the lateral semicircular canals. The same durations were used for the vertical canals. Stimuli were 4 /sec² accelerations and decelerations separated by 54 sec of constant velocity for cats and by 150 sec of constant velocity for humans. In cats, vertical canal stimulation was accomplished by placing each animal on its right side to locate its sagittal plane at the center and in the plane of rotation. human subjects leaned forward with the head turned to place the sagittal plane of the skull in the plane (and at the center) of rotation. A biteboard and head rest assisted in this positioning. Stimuli were presented in a counterbalanced order as indicated in Table 1

Neither the cats nor the human subjects had been used in previous vestibular experiments. For the humans, this necessitated instruction regarding the signalling of subjective events without actual practice in making such judgments. they reported onset and cessation of apparent rotation by means of a signal key

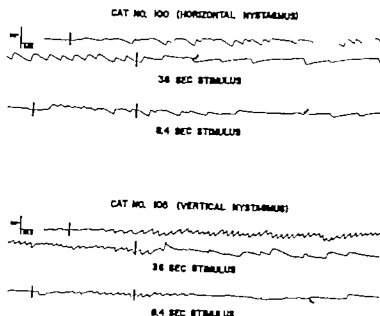


FIG. 1 Nystagmus recorded from two cats fo two durations of a 4 /sec² ngular accelera tion. Responses to stimulation of the lateral canals (Cat No 100) and the ertical canal (Cat No 103) are presented. V rtical bars through the tracing demarcate the stimulus periods rrows indicate the start of secondary nystagmus. Note the l ng post timulus primary nyst gmu and the later onset of econdary nyst gmus fo the sh rter duration of acceleration.

RESULTS AND DISCUSSION

Cats

The 8 animals received only 4 trials (2 lateral and 2 vertical canal stimulations) on the first day Some examples of recorded nystagmus appear in Fig 1 The critical portion of the response for several purposes of this study began at the *end of each stimulus* Thus, time measurements were made from the end of each stimulus (*a*) to the end of the primary response and (*b*) to the start of the secondary nystagmus. The number of beats of primary nystagmus which followed stimulus termination was also tabulated These data appear in Table 2 In 47 of the 48 comparisons, the primary post stimulus responses to the 8 4 sec stimulus exceeded those of the 36 sec stimulus.

Plots of the complete nystagmic responses to the 8 4 and the 36 sec stimuli appear in Fig 2 Slow phase output was scored by summing the vertical displacements of beats from peak to base line for successive 3-sec intervals. Greater output of primary nystagmus is evident for the lateral canals, as compared with the vertical canals, for both stimulus durations. For the 8.4 sec timuli, primary nystagmus increased throughout the stimulus period for both the horizontal and "vertical curves. For the 36 sec stimulus, there was a marked peaking in the response t stimulati n of the vertical canals during the 15–18 sec interval and a steady decline of that response throughout the remainder of the stimulus. For stimulation

TABLE 2. *Measures of primary nystagmus following the termination of each rotatory stimulus for cats*

Each response value is mean of responses to acceleration and deceleration stimulus. Stimuli were 4°/sec² for either 8.4 or 36 seconds.

	Time from end of stimulus to end of primary nystagmus (sec)				Time from end of stimulus to start of secondary nystagmus (sec)				Beats of primary nystagmus after end of stimulus			
	Lateral		Vertical		Lateral		Vertical		Lateral		Vertical	
Cat	8.4	36	8.4	36	8.4	36	8.4	36	8.4	36	8.4	36
100	9.2	2.5	6.5	0.5	12.9	6.7	11.1	3.4	8.8	2.8	9.0	1.0
101	11.4	7.0	7.9	7.0	14.4	8.7	9.1	9.4	14.8	10.0	11.5	7.8
102	3.2	0.5	23.7	5.7	6.0	0.9	28.3	9.1	3.3	0.5	18.5	4.0
103	4.9	4.4	8.8	4.2	8.1	6.3	18.1	6.2	3.5	1.5	7.5	3.5
104	10.7	8.4	8.2	2.1	12.0	6.6	10.9	3.9	8.8	2.0	7.0	2.5
105	13.8	4.9	13.6	2.0	15.8	10.4	14.6	4.3	14.5	8.0	10.0	2.8
106	11.5	5.7	8.0	4.8	14.7	7.0	7.7	7.2	10.0	4.8	8.3	4.5
107	10.8	8.2	6.0	−1.5*	14.6	11.0	17.5	2.7	7.5	6.0	6.5	1.0
M	9.5	5.1	10.3	3.4	12.3	7.1	14.7	5.9	8.9	4.4	9.4	3.4

* Nystagmus ended during stimulus.

of the lateral canals during the 36 sec stimulus, peaking occurred in the 18–21 sec interval and was followed by a lesser decline than that noted for the vertical canals.

Secondary nystagmus was also plotted in Fig. 2. With only one exception (the 8.4 sec stimulus to the vertical canals for cat No. 100) clear and quantifiable secondary nystagmus was obtained from each cat for each stimulus condition. For both vertical and lateral canal responses, the 36 sec stimuli produced greater secondary responses than did the 8.4 sec stimuli. In addition, horizontal secondary nystagmus showed greater output than vertical secondary nystagmus by amounts proportional to differences in their respective primary reactions. Further, the mean peak of the secondary response occurred 21–24 sec after the end of the 8.4 and 36 sec stimuli for horizontal nystagmus, and 15–21 sec after the 8.4 and 36 sec stimuli for vertical nystagmus.

To pursue further the relationship of secondary to primary nystagmus and the effect of prolonged stimuli on those responses, six of the animals were given, one day later, a series of 16 trials stimulating the lateral canals with the 4°/sec² stimulus for 36 sec duration. Tracings for trials 1, 5, 10 and 15 were scored and the data plotted in Fig. 3. With repeated stimulation, a marked depression of both the primary and secondary response curves occurred, peaking of the response was followed by a decline in nystagmic output during the remainder of the stimulus, and the peaks of both primary and secondary nystagmus shifted toward earlier occurrences (see Collins, 1964).

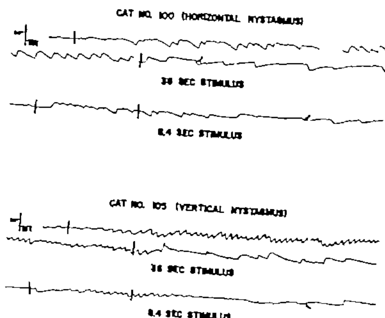


FIG. 1 Nystagmu recorded from tw cats for two durations of a 4 /sec² angular acceleration Responses to stimulation of the lateral canals (Cat No 100) and the ertical canals (Cat No 105) are presented Vertical bars through the tracing dem rcate the stimulus periods arrows indicate the tart of secondary nystagmu N te the l nger poststimulu primary nyst gmus and the later onset of secondary nystagmus for the shorter duration of accel ration

RESULTS AND DISCUSSION

Cats

The 8 animals received only 4 trials (2 lateral and 2 vertical canal stimulations) on the first day Some examples of recorded nystagmus appear in Fig 1 The critical portion of the response for several purposes of this study began at the *end of each stimulus* Thus, time measurements were made from the end of each stimulus (*a*) to the end of the primary response and (*b*) to the start of the secondary nystagmus. The number of beats of primary nystagmus which followed stimulus termination was also tabulated These data appear in Table 2 In 47 of the 48 comparisons, the primary post stimulus responses to the 8 4 sec stimulus exceeded those of the 36 sec stimulus.

Plots of the complete nystagmic responses to the 8 4 and the 36 sec stimuli appear in Fig 2 Slow phase output was scored by summing the vertical di placements of beats from peak to base line for successive 3-sec intervals. Greater output of primary nystagmus is evident for the lateral canals, as compared with the vertical canals, for both stimulus durations For the 8 4 sec stimuli primary nystagmus increased throughout the stimulus period for both the "horizontal" and "vertical" curves. For the 36 sec stimulus, there was a marked peaking in the resp nse to stimulation of the vertical canals during the 15–18 sec interval and a steady decline of that response throughout the remainder of the stimulus For stimulation

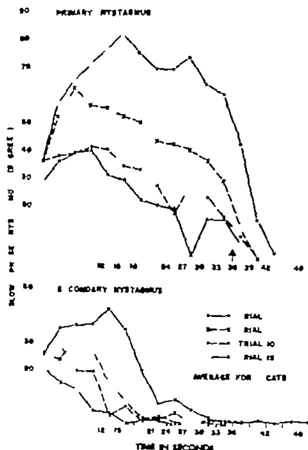


Fig. 3. Time-course plot of slow-phase eye displacement for trials 1, 5, 10, and 15 of the habituation series. With repeated trials there occurs depression of output with early peaking followed by rapid declines during the decelerations. Effects are similar for both primary and secondary nystagmus.

turning was calculated from the *end of each stimulus* to the end point of the sensation (Table 4).

In 20 of the 48 comparisons (Table 3) human responses to the 38 sec stimulus exceeded responses to the 8.4 sec stimulus (9 of these cases were for the "number of beats" measure) and, although the mean group data for duration of primary nystagmus and for "time from end of stimulus to start of secondary nystagmus" were longer for the 8.4 sec stimulus, the differences were slight. Mean number of beats of primary nystagmus following stimulus termination actually favored the 38 sec over the 8.4 sec stimulus, but the differences were not significant. Thus, results obtained from the cats, in which the post stimulus nystagmic responses to the 8.4 sec stimulus consistently exceeded those of the 38 sec stimulus, were not borne out in the data from human subjects.

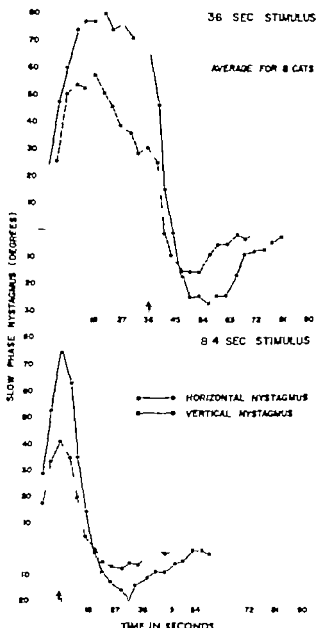


Fig. 2. Time-course plot of slow-phase eye displacement in 3-sec intervals for 8 cats. Arrows indicate termination of acceleration. Points above the zero line represent primary nystagmus; points below the zero line represent secondary nystagmus. In the upper graph, clear peaking and decline of nystagmus during the acceleration is evident for response to both lateral and vertical-canal stimulation. In the lower graph (8.4 sec stimulus), 3-sec intervals were marked off from the end of the stimulus (the first 3-sec interval represents response during only 2.4 sec of stimulus). Total output of both primary and secondary nystagmus is consistently greater for the lateral canals.

Human Subjects

Eight human subjects (4 males and 4 females) were given stimulations identical to those administered to the cats. Nystagmus data were also scored similarly and appear in Table 3. Some examples of nystagmus tracings are presented in Fig. 4. In addition, duration of the sensation of

TABLE 4 *Time in seconds from end of each rotatory stimulus to end of subjective turning experience for human subjects*

Each line l meta for acceleration nd a deceleratio stimulus. Stimuli were 4°/sec² for either 8.4 or 36 seconds.

Subject	Lateral canals 8.4	Lateral canals 36	Vertical canals 8.4	Vertical canals 36
N	—	—	—	—
Or	—	29.5	12.5	9.3
Me	34.5	12.4	44.0	9.2
W	27 8	14.9	10.4	23.7
Fu	11.0	6.7	7 7	2 0
F	23.1	14.3	11.6	28.6
Jo	20 4	12.4	19.9	−1.3[a]
Ma	7 7	3.1	7 4	−15.1[a]
M	20.8	13.3	16.2	8.1

[a] Subjectiv t rning xperience ended d ri g stimulus.

Human subjective data (Table 4) in 11 of 13 comparisons (3 comparisons were n t obtained) showed that the 8 4 sec stimulus resulted in sensations of longer duration after termination of angular acceleration than did the 36 sec stimulus. F r two subjects, the sensation to vertical canal stimula tion ended during the 36 sec stimulus.

Time plots of the nystagmus recorded during the two stimulus conditions appear in Fig. 5 Responses from the lateral canals were of greater magnitude than those from th vertical canals. No clear peaking or decline in output during the stimulus appeared f r either set of canals. Secondary nystagmus was plotted in the same figure Although an apparent starting point could u ually be ascertained for secondary responses, the nystagmus was frequently either very weak and brief or very unclear Thus, quantifi able secondary responses were obtained from only 2 of the 8 subjects for the 8.4 sec lateral canal stimulus, from only 3 for the same stimulus applied t the vertical canals, from 5 for the 36 sec vertical canal stimulus, and from 7 subject for the 36 sec l teral canal timulus. In two general respects, then human data differed from cat data (1) human did not show a rise and decline during prolonged (36 sec) stimulation whereas the cats did (2) little or n secondary nystagmic reaction to the 8.4 sec stimuli were demon strated by human whereas cats consistently ga e such responses.

Arousal

Th import nce f arousal on nystagmi responses was noted earlier and ha been examined in con iderable detail elsewhere (Collins, 1962) To e sure r li bility of the present data with regard to this factor a second group of 10 cats wa treated with *d*-amphetamine in accordance with procedures described by Crampton & Brown (1964) Each animal received

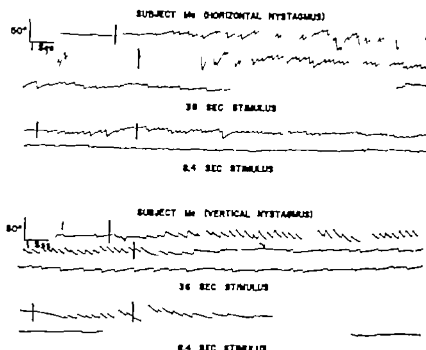


FIG 4 Nystagmus recorded from two human subjects fo two duratio s of a 4 /sec² angular acceleration Respon es t stimulation of th l teral canal (Subject M) and of the vertical canals (Subject Me) are presented M rkings are the same as in Fig. 1 The learly longer responses of the lateral canals to the shorter timulus durati n which were obtained from cats are not o evident here (compare Subject M data with Fig 1) Subject M (ertical nystagmu) was typical of human bjects in dem strating clear d con ist nt secondary nystagmus with tra ings lo ly resembling those obtained from cats.

TABLE 3 *Measures of primary nystagmus following the termination of each rotatory stimulus for human subjects*

Ea h re ponse v lue is a mean of response to an cceleratl n nd deceleration tim l s Stimuli were 4 sec² for eithe 8 4 36 seconds.

	Time from end of stim lus to end of primary nystagmus (sec)				Time from end of timulus to tart of secondary nyst gmus (sec)				Beat of prim ry ny tagmus after end of stim lus			
	Lateral		Vertical		Lateral		Vertical		Lateral		Vertical	
Subject	8.4	36	8.4	36	8.4	36	8.4	36	8.4	36	8.4	36
Nu	21.7	18.9	21.1	7.6	32.9	26.5	[illegible]	13.4	18.5	14.5	15.0	9.0
Or	29.8	34.7	16.1	26.5	32.0	36.0	9.8	0.0	92.0	[illegible]	25.5	[illegible]
M	32.0	4	10.0	14.6	35.6	32.1	21.2	16.5	[illegible]	21.5	13.0	9.5
Wa	42.1	33.0	8.5	10.5	45.1	38.6	13.6	14.9	23.0	32.5	5.0	10.5
Pe	18.2	23.2	6.2	11.2	21.6	21.5	0.8	10.2	0.5	21.0		9.5
Fr	30.2	30.4	22.4	9.0	39.8	30.1	[illegible]	14.5	35.5	45.5	16.0	13.0
J	37.1	36.1	15.8	4.0	36.6	32.6	—	8.0	36.0	47.5	[illegible]	[illegible]
Ma	41.0	35.8	9.4	13.7	41.4	13.0	20.2	17.1	51.5	[illegible]	[illegible]	[illegible]
M	32.7	30.1	14.4	12.3	36.4	33.0	21.2	14.1	[illegible]	40.6	11.4	11.5

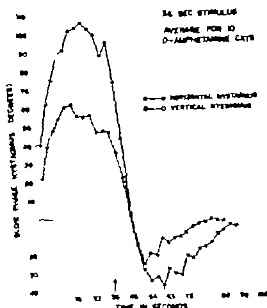
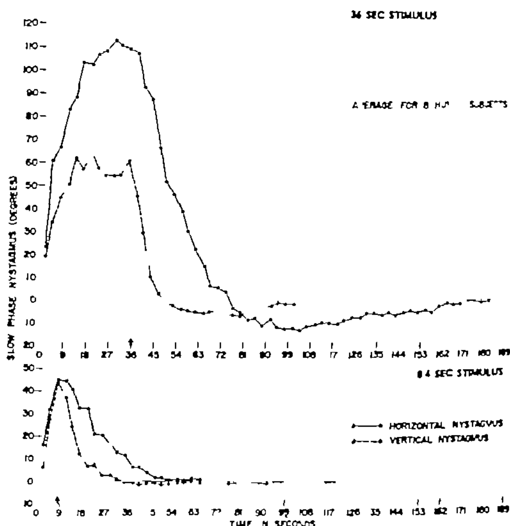


Fig. 6. Time-course plots of primary and secondary nystagmus in 3-sec intervals for cats treated with d-amphetamine. Similar to results obtained from undrugged cats, peaking and decline of the response during the 36 sec stimulus (4°/sec²) is evident for both lateral and vertical-canal stimulation (compare with Fig. 2).

(1966) who showed plots of single neural unit activity during a prolonged angular acceleration and reported a rise and decline of firing during the stimulus period.

A reliability check of the findings for humans was accomplished by exposing 4 males and 4 females, all previously untested, to stimulus conditions (4°/sec² for 36 sec) identical to those administered to the other human subjects. Half of the males and females received CW stimulation, the remaining half received CCW rotation. Four trials were administered, two each for the lateral and vertical canals. The first two trials always employed mental arithmetic (MA) as an arousal task (see Collins, 1962), while in the last two trials, the Key Press (KP) technique of estimating subjective velocity was used to maintain alertness. With the KP technique the subjects attempted to signal successive angular displacements of 90°. Table 5 contains an outline of the test procedures for this reliability check. Nystagmus data for acceleration and deceleration and for the two tasks showed no evidence of a fall-off in response during stimulation, and the primary-to-secondary nystagmus transition points were almost identical to those obtained under the first set of conditions. The data were thus combined and curves depicting horizontal and vertical nystagmus were plotted in Fig. 7. As in the first group of human subjects, no clear evidence for a decline of response during the stimulus is evident. However, the vertical nystagmus time plot does show considerable irregularity as compared with horizontal



Fro 5 Time-course pl ts of slow phase eye displacem nt in 3-sec interval f r 8 huma ubjects. M rki gs re the sam as in Fig. 2 Tot l utput f both p imary nd secondary nyst gmu l greater f r th l teral canals In the upper graph, no clear peak! g and ubsequ nt decline of nystagmus during the cceleration is evident, lthough a large dip ppears in the dat f the vertical canal (compare with Fig. 7)

4 trials with each trial comprising an acceleration stimulus of 4 /sec² for 30 sec 2 min of constant velocit and a sub threshold deceleration (0 15 /sec²) The first two trials were always stimulation of the lateral canals the remaining two trials involved vertical canal stimulation Trials were alternatel CW and CCW Half the animals began with CW rotation the remaining 5 began with CCW rotation Data from CW and CCW ac celerations were similar for the horizontal and the vertical nystagmus curves and, theref re were averaged The mean response curves for the 10 animals appear in Fig 6 and demonstrate the same type of decline during stimulation and almost identical transition points from primary to secondary nystagmus as those presented b the undrugged cats Vertical nystagmus again shows a more pronounced decline during stimulation than does horizontal nystagmu Some supportive neural data for this decline of response during stimulation of the cat has been presented b Cappel

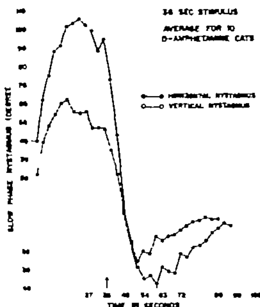


Fig. 6 Time-course plot f primary and secondary nystagm i 3-sec intervals for cats treated with d-amphetamine Similar t results btained from ndrugged cats, peaking nd decline of the response during the 36 sec stim lus (4 sec²) i evident for both lateral- nd ertical-ca al stimulation (compare with Fig. 2)

(1966) who showed plots of single neural unit activity during a prolonged angular acceleration and reported a rise and decline of firing during the stimulus period

A reli bility check of the findings for humans was accomplished by ex posing 4 males and 4 females, all previously untested, to stimulus conditions (4 /sec² for 30 sec) identical to those administered to the other human subjects. Half of the males and females received CW stimulation, the remaining half received CCW rotation. Four trials were administered, two each f r the lateral and vertical canals. The first two trials always employed mental arithmetic (MA) as an arousal task (see Collins, 1962) while in the last two trials, the Key Press (KP) technique of estimating subjecti e elocity wa used t maint in alertness. With the KP technique the subjects ttempted t ignal ucces i e angular displacements of 90 Table 5 con tain an outline f the test procedures for thi reliability check. Nystagmu data for acceleration and deceleration and for the two tasks showed no evidence f a fall-off in response during stimul tion and the prim ry to-second ry nystagmus transition points were almost identical to those obtained under the first set of conditions. The data were thu combined and curves depicting horizontal and vertical nystagmus were plotted in Fig 7 A in the first group of human subjects, no clear evidence for a decline of response during the stimulus is evident. However the vertical nystagmus t me plot does show consid rable irregularity as compared with horizontal

TABLE 5 *Order of stimulus presentation for human subjects used in the reliability check*

Arousal of subjects was controlled by Mental Arithmetic and Key Press tasks. All trials comprised accelerations and decelerations of 4 /sec² for 36 sec separated by 2 min of constant velocity

Subjects		Rotation direction	Mental arithmetic		Key press	
Male	Female		Trial 1	Trial 2	Trial 3	Trial 4
A	W	CW	Lateral	Vertical	Vertical	Lateral
B	X	CCW	Lateral	Vertical	Vertical	Lateral
C	Y	CW	Vertical	Lateral	Lateral	Vertical
D	Z	CCW	Vertical	Lateral	Lateral	Vertical

nystagmus data Thus, the differences between man and cat that were obtained from the first groups of subjects were confirmed with different subjects and under conditions in which the arousal variable was manipulated

It has been reported that vestibular nystagmus rises and declines, like the subjective reaction during constant angular acceleration (Buys, 1924 Mowrer 1935 Wendt 1950 Hood 1961) However Guedry & Lauver (1961) demonstrated that the nystagmic reaction in man did not decline during prolonged constant angular acceleration if the subjects were required to signal estimates of angular displacement Occasionally however a subject would yield a rise and decline in the nystagmus response similar to the subjective responses in earlier experiments (see Fig 4 in Guedry & Lauver 1961) To check the possibility that arousal accounted for the variety of findings, Collins & Guedry (1962) required subjects, during prolonged angular accelerations, to perform mental tasks, which would maintain mental activity independent of the subjective perception of rotation Subjects were required to make arithmetic computations throughout the vestibular stimulation and post stimulation periods. Results showed again that nystagmus first increased and then remained constant during constant angular acceleration Following the termination of stimulation, nystagmus decayed about as expected from the "torsion pendulum" theory (Groen 1960) although rates of decay were not calculated The same subjects, when allowed to relax, occasionally showed a rise and decline of nystagmus during constant stimulation and a rapid decay of response on termination of the stimulus. The present experiments confirm the fact that nystagmus does not decline during prolonged angular acceleration in alert human subjects, although such declines appear to occur in cats.

In cats, time to *onset* of secondary nystagmus was inversely related to duration of the stimulus. The earlier *onset* of the secondary reaction cannot be attributed to loss of arousal This result strongly suggests that the decline in primary nystagmus during and after the longer stimuli resulted

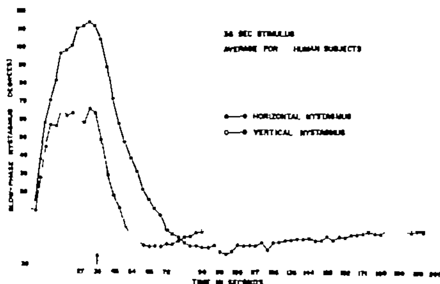


FIG. 7 Time-course plot f primary nystagmus I 3-sec int rval for second group of humans he were given instructions f fluenci g arousal duri g the tests. Stimuli were 4°/sec² angular acceleration for 36 sec. Ea h poi t i an erag of two task-trial (mental arithmetic and key pressi g) for ach of the 8 subjects. Simila t results obtained under other arousal conditions (compare with Fig. 5) as peaking and subsequent decline of nystagm during accelerations is evident. One female subject wa excluded from the erage curves due t the presence f spontaneou ystagmus which particularly affected scoring of secondary responses.

from a counteracting process which reduced the intensity and duration of the primary nystagmus and then became evident as an early secondary nystagmus. Data from humans have shown that as the duration of a con stant angular acceleration is increased beyond certain time limits, the dura tion of the subjective after-responses becomes shorter and shorter (Guedry & Beberman, 1957 Guedry Cramer & Koella, 1958) Nystagmic reactions in cats showed similar effects and, in these respects, more closely resembled the subjective reactions of man than they did the nystagmus of man

ACKNOWLEDGMENTS

This study wa conducted t the Civil Aeromedical Institut I Oklahoma City with the technical assistance f Billy P Updegraff Ruth An Mertens, nd Kathry Quattlebaum.

RESUME

Enregistrements de nystagmus oculaire furent obtenus vec des hats et des hommes qui furent exposés aux accélérations angulaires de 4 /sec² d'inte sité et des durées d 8,4 et d 36 sec. Les can u latérau et les ca u verticau

TABLE 5 *Order of stimulus presentation for human subjects used in the reliability check*

Arousal of subjects was controlled by Mental Arithmetic and Key Press tasks. All trials comprised accelerations and decelerations of 4 /sec² for 36 sec separated by 2 min of constant velocity

Subjects		Rotation direction	Mental arithmetic		Key press	
Male	Female		Trial 1	Trial 2	Trial 3	Trial 4
A	W	CW	Lateral	Vertical	Vertical	Lateral
B	X	CCW	Lateral	Vertical	Vertical	Lateral
C	Y	CW	Vertical	Lateral	Lateral	Vertical
D	Z	CCW	Vertical	Lateral	Lateral	Vertical

nystagmus data Thus, the differences between man and cat that were obtained from the first groups of subjects were confirmed with different subjects and under conditions in which the arousal variable was manipulated

It has been reported that vestibular nystagmus rises and declines, like the subjective reaction, during constant angular acceleration (Buys, 1924 Mowrer 1935 Wendt, 1950 Hood 1961) However Guedry & Lauver (1961) demonstrated that the nystagmic reaction in man did not decline during prolonged constant angular acceleration if the subjects were required to signal estimates of angular displacement Occasionally however a subject would yield a rise and decline in the nystagmus response similar to the subjective responses in earlier experiments (see Fig 4 in Guedry & Lauver 1961) To check the possibility that arousal accounted for the variety of findings, Collins & Guedry (1962) required subjects, during prolonged angular accelerations, to perform mental tasks, which would maintain mental activity independent of the subjective perception of rotation Subjects were required to make arithmetic computations throughout the vestibular stimulation and post stimulation periods. Results showed again that nystagmus first increased and then remained constant during constant angular acceleration Following the termination of stimulation, nystagmus decayed about as expected from the "torsion pendulum" theory (Groen 1960) although rates of decay were not calculated The same subjects, when allowed to relax, occasionally showed a rise and decline of nystagmus during constant stimulation and a rapid decay of response on termination of the stimulus. The present experiments confirm the fact that nystagmus does not decline during prolonged angular acceleration in alert human subjects, although such declines appear to occur in cats.

In cats, time to *onset* of secondary nystagmus was inversely related to duration of the stimulus. The earlier *onset* of the secondary reaction cannot be attributed to loss of arousal This result strongly suggests that the decline in primary nystagmus during and after the longer stimuli resulted

REFERENCES

Buys, E., 1924 Contribution à l'étude du nystagmus oculaire de la rotation chez l'homme. *Rev Otoneuroophtal.*, 2 441

Carter, K. L., 1966 Determination of physical constants of the semicircular canals from measurement of single neural unit activity under constant angular acceleration. In *Second Symposium on the Role of the Vestibular Organs in the Exploration of Space* NASA Report SP 115, 229

Collins, W. E., 1962 Effect of mental set upon vestibular nystagmus. *J Exp. Psychol* 63, 191

— 1964 Primary secondary and caloric nystagmus of the cat following habituation to rotation. *J Comp. Physiol Psychol* 57 417

Collins, W. E., and Guedry, F. E., 1962 Arousal effect and nystagmus during prolonged constant angular acceleration. *Acta Otolaryng* (Stockh.) 54 349

Collins, W. E., and Huffman, H. W. 1964 Design and performance characteristics of mechanically driven vestibular stimulator *OAM Report No.* 64–15, Oklahoma City Oklahoma

Collins, W. E., and Updegraff, B. P. 1966 A comparison of nystagmus habituation in the cat and the dog. *Act Otolaryng* (Stockh.) 62 19

Crampton, G. H., and Brown, J. H., 1964 Repeated vertical semicircular canal stimulation does not habituate horizontal nystagmus in cat. *Act Otolaryng* (Stockh.) 58, 441

Dodge, R., 1923 Habituation to rotation *J Exp Psychol* 6, 1

Ek, J., Jongkees, L. B. W. and Klijn, J. A. J. 1960: On the effect of continuous acceleration. *Act Otolaryng* (Stockh.) 51 416.

Groen, J. J. 1960 Problems of the semicircular canal from a mechanicophysiological point of view *Acta Otolaryng* (Stockh.) 3 ppl. 163 59

Guedry, F. E., and Beckman, N. 1957 Apparent adaptation effects in vestibular reactions *U.S. Army Med. R. a. Lab. Report No.* 293, Fort Knox, Kentucky

Guedry, F. E., and Ceran, S. J. 1959 Derivation of subjective velocity from angular displacement estimates made during prolonged angular accelerations. Adaptation effects. *U.S. Army Med. Res Lab. Report* No. 376, Fort Knox, Kentucky

Guedry, F. E., Cramer, R. L., and Koella, W. P. 1958 Experiments on the rate of development and the rate of recovery of apparent adaptation effects in the vestibular system. *U.S. Army Med. Res. Lab Report No.* 338, Fort Knox, Kentucky

Guedry, F. E., and Lauver, L. S., 1961 *Vestibular nystagmus* during constant angular acceleration. *J Appl. Physiol* 16 215

Henriksson, N. G., Fernandez, C., and Kohut, R. L. 1961 The caloric test in the cat. *Act Otolaryng* (Stockh.) 53, 71.

Hood, J. D. 1961 The neuro-physiological significance of cupular adaptation and its bearing upon Ewald's second law. *Acta Otolaryng* (Stockh.) Suppl. 159 50

Mittermaier, R. and Rossberg, G. 1956 Vestibularuntersuchungen mit schwellennahen Beschleunigungsreizen *Arch. Ohr Nas. Kehlk. Heilk.*, 168, 313.

Montandon, A., and Russbach, J. 1957 Valeur diagnostique comparative des nystagmus pre- et post-rotatoires à l'épreuve giratoire liminaire *Pract Ot. Rhinolaryng.* (Basel) 19 556

Mowrer, O. H. 1935 The nystagmic response of the pigeon to constant angular acceleration at liminal and supraliminal intensities. *J Comp Psychol* 19 177

Wendt, G. R., 1950 Some characteristics of vestibular eye movements. In *Psychophysiological Factors in Spatial Orientation*, US ONR Symposium, U.S. Naval Air Station, Pensacola, Florida.

AC 118, CAMI-FAA Box 25082
Oklahoma City Oklahoma 73125 U.S.A.

Received March 15, 1967

furent stimulés avec des accélérations indépendantes. Les données montrent que le nystagmus primaire et le nystagmus secondaire furent l'un et l'autre plus elevés avec les canaux latéraux qu'avec les canaux verticaux. Chez les chats, les réponses des canaux latéraux et des canaux verticaux pour la stimulation d'une durée de 36 sec atteignirent tous les deux au plus haut point après 15–21 sec d'accélération angulaire et ensuite se baissèrent régulièrement. Ces déclins ne se manifestèrent pas avec les réponses des hommes. En un épreuve additionnelle les hommes exécutèrent des tâches particulieres et les chats reçurent de d-amphetamine. On obtenit les mêmes données que celles décrites par-dessus. Des autres différences entre les deux groupes furent observées. Les chats démontrèrent conformément du nystagmus secondaire tandis que les hommes n'en démontrèrent pas conformément. Après la terminaison d'accélération le nystagmus primaire des chats dura plus long et démontra plus de mouvements des yeux suivant les stimuli d'une durée de 8,4 sec que suivant les stimuli d'une durée de 36 sec; cette consistance ne fut pas évidente chez les hommes. Cependant, chez les hommes les sensations de mouvement suivant la terminaison d'accélération durèrent plus long après les stimuli d'une durée de 8,4 sec qu'après les stimuli d'une durée de 36 sec. A ce sujet le nystagmus des chats ressemblèrent plus aux réactions subjectives des hommes qu'au nystagmus des hommes.

ZUSAMMENFASSUNG

An einer Gruppe von Katzen und parallel dazu an einer Gruppe von Versuchspersonen wurde der okulare Nystagmus bei Erteilung einer Winkelbeschleunigung von 4°/sek² von 8,4 sek Dauer und von 36 sek Dauer registriert. Die Reizung der lateralen Bogengänge und der vertikalen Bogengänge wurde dabei in separaten Versuchen vorgenommen. Die Untersuchungen ergaben, dass sowohl der Primär- wie der Sekundärnystagmus grösser war bei Reizung der lateralen Bogengänge. Weiterhin zeigten die Versuche an den Katzen, dass die Reaktion während der 36-sek Reize, und zwar sowohl bei Reizung der lateralen Bogengänge wie bei Reizung der vertikalen Bogengänge, nach 15–21 sek angulärer Beschleunigung ein Maximum erreichte und danach stetig abnahm. Bei der menschlichen Gruppe dagegen war keine Abnahme des Nystagmus zu vermerken. In weiteren Untersuchungen wurden den Versuchspersonen während der Stimulationsphasen bestimmte Aufgaben gestellt, während den Katzen in den Parallelversuchen d-Amphetamin verabfolgt wurde. Im wesentlichen ergaben sich dabei die gleichen Resultate wie bei den obigen Versuchen. An weiteren Unterschieden zwischen den beiden Versuchsgruppen war zu verzeichnen, dass die Katzen konsequent Sekundärnystagmus zeigten, während er bei den Versuchspersonen nicht die Regel war. Auch hielt der Primärnystagmus nach Beendigung der Beschleunigung bei den Katzen nach dem 8,4-sek Reiz länger an, und die Zahl der Augenbewegungen war grösser als nach dem 36-sek Reiz. Bei den Versuchspersonen dagegen war in dieser Hinsicht keine ähnliche regelmässige Reaktion feststellbar. Andererseits hielt bei den Versuchspersonen der subjektive Eindruck der Bewegung nach Beendigung der Beschleunigung nach dem 8,4-sek Reiz länger an als nach dem 36-sek Reiz. In letzterer Hinsicht ähnelte also der Nystagmus bei den Katzen mehr der subjektiven Reaktion beim Menschen als dem Nystagmus beim Menschen.

tion of the opto-kinetic nystagmus was enhanced by the experimental work of Ter Braak (1936) which showed the existance of two distinct types of opto-kinetic nystagmus. He designated one the cortical or "look" nystagmus, and the other subcortical or "stare" nystagmus. While both exist in man it is believed that only the subcortical type is present in most animals. In some instances the cortical type of opto-kinetic nystagmus was elicited in animals if the opto-kinetic stimulus was such as to produce a strong motivation upon the animal (i.e., rabbit rotated around a dog)

The nervous centers involved in the production of the opto-kinetic nystagmus were identified by earlier investigators from observations made on clinical cases. The organization of the reflex arc as described by Cords (1926) is generally accepted, however the precise neural pathways remain unknown and the physiological role performed by the different parts is still controversial A comprehensive review of the subject was published by Bender (1964)

Ehlers (1926) Gruttner (1939) Mackensen (1954) and Enoksson (1956) observed in man, that the frequency of the opto-kinetic nystagmus increased as the velocity of the opto-kinetic stimulus was increased up to a certain point beyond which, the frequency of the opto-kinetic nystagmus assymtotically reached a steady state of constant frequency This usually occurred at stimulus velocities of 50 to 70 degrees/sec. With further increase of velocity the frequency of the opto-kinetic nystagmus declined and at times disappeared

Ehlers (1926) was the only investigator to use sufficient numbers of small increments in velocity of the opto-kinetic stimuli to enable recognition of the close relationship between the frequency of the nystagmus and the velocity of the stimulus. He concluded from visual observations that opto-kinetic nystagmus showed great physiological constancy This work was extended by Enoksson (1956) Gruttner (1939) and Mackensen (1954) who studied not only the frequency of the opto-kinetic nystagmus but also the velocity of the slow component for different opto-kinetic stimuli. Their observations were done mostly during the steady state value of the frequency of the nystagmus using large opto-kinetic stimulus velocities. Therefore no precise relationships could be established between the opto-kinetic stimulus and the nystagmus parameters.

In the cat we have studied the characteristics of the optically induced nystagmus produced by rotating a drum at different velocities around the animal Electronystagmographic techniques were utilized to obtain quantitative measurements for the assessment of opto-kinetic nystagmus

METHOD

Young healthy cats were anesthetized with Nembutal given intraperitoneally (0 7 cc per kilo) and permanent electrodes were implanted. A rectangular 5 × 10 mm lucite platform 1 mm thick was fixed to the skull

Acta oto-laryngologica 64 335–40 (1967)

EXPERIMENTAL STUDIES ON OPTOKINETIC NYSTAGMUS

I. Normal Cats

V. Honrubia, Barbara J. Scott and P. H. Ward
Nashville, Tenn., U.S.A.

From the Division of Otolaryngology and the Bill Wilkerson Hearing and Speech Center of Vanderbilt University School of Medicine, Nashville

Opto-kinetic nystagmus in normal cats was studied under a variety of conditions. The relationships between the various parameters of nystagmus and the opto-kinetic stimulus were consistently stable throughout the repeated testing for periods up to seven months. The frequency of the opto-kinetic nystagmus was primarily dependent on the velocity of the opto-kinetic stimulus and independent of the number of stripes placed on the wall of the opto-kinetic drum. The frequency, amplitude and the velocity of the slow component of nystagmus increased with increase in the velocity of the opto-kinetic drum until a maximum was reached at stimulus velocities of 40 to 50 degrees/sec. The velocity of the eye during the slow component of nystagmus is not constant. It has an exponential course, the velocity becoming progressively smaller during each nystagmic beat. The duration of both the fast and slow components diminished with increase in the frequency of nystagmus. The physiological significance of these measurements is emphasized.

The slow component of the opto-kinetic nystagmus has been considered as a pursuing movement of the eyes in an effort to keep the moving objects in a fixed zone of clear vision on the retina (Dodge & Fox, 1928; Rademaker & Ter Braak, 1948). The conclusion was drawn that the velocity of the slow component of the opto-kinetic nystagmus is equal or very close to the velocity of the visual stimuli that produced it. Based on this assumption the opto-kinetic nystagmus has been used as a reference calibration for measurements of the velocity of the slow phase of vestibular nystagmus. Quantitative studies of the relationships between the values of the different components of the nystagmic beat produced at different velocities of the opto-kinetic stimuli could not be found (i.e., amplitude, duration and velocity of the slow components, duration and velocity of the fast components and frequencies of the opto-kinetic nystagmus). A study of this kind seemed desirable to enable more accurate evaluation of the experimental studies of nystagmus.

Our knowledge of the neurological mechanism underlying the produc-

This work was supported in part by United States Public Health Service Grant NB 3350-01 and NB 03674-03

tion of the opto-kinetic nystagmus was enhanced by the experimental work of Ter Braak (1936) which showed the existance of two distinct types of opto-kinetic nystagmus. He designated one the cortical or "look" nystagmus, and the other subcortical or "stare" nystagmus. While both exist in man it is believed that only the subcortical type is present in most animals. In some instances the cortical type of opto-kinetic nystagmus was elicited in animals if the opto-kinetic stimulus was such as to produce a strong motivation upon the animal (i.e. rabbit rotated around a dog)

The nervous centers involved in the production of the opto-kinetic nystagmus were identified by earlier investigators from observations made on clinical cases. The organization of the reflex are as described by Cords (1926) is generally accepted, however the precise neural pathways remain unknown and the physiological role performed by the different parts is still controversial. A comprehensive review of the subject was published by Bender (1964)

Ehlers (1926) Gruttner (1939) Mackensen (1954) and Enoksson (1956) observed in man, that the frequency of the opto-kinetic nystagmus increased as the velocity of the opto-kinetic stimulus was increased up to a certain point beyond which, the frequency of the opto-kinetic nystagmus assyntotically reached a steady state of constant frequency This usually occurred at stimulus velocities of 50 to 70 degrees/sec. With further increase of velocity the frequency of the opto-kinetic nystagmus declined and at times disappeared.

Ehlers (1926) was the only investigator to use sufficient numbers of small increments in velocity of the opto-kinetic stimuli to enable recognition of the close relationship between the frequency of the nystagmus and the velocity of the stimulus. He concluded from visual observations that opto-kinetic nystagmus showed great physiological constancy This work wa extended by Enoksson (1956) Gruttner (1939) and Mackensen (1954) who studied n t only the frequency of the opto-kinetic nystagmus but also the velocity of the slow component for different opto-kinetic stimuli. Their observations were done mostly during the steady state value of the frequency of the nystagmus using large opto-kinetic stimulus velocities. Theref re no precise relati nships could be established between the opto-kinetic stimulus and the nystagmus parameters.

In the cat we have studied the characteristics of the optically induced nystagmus produced by rotating a drum at different velocities around the animal. Electronystagmographic techniques were utilized to obtain quantitative measurements for the assessment of opto-kinetic nystagmus.

METHOD

Young healthy cats were nesthetized with Nembutal given intraperitoneally (0 7 cc per kilo) nd permanent electrodes were implanted. A rectangular 5 × 10 mm lucite platform 1 mm thick was fixed to the skull

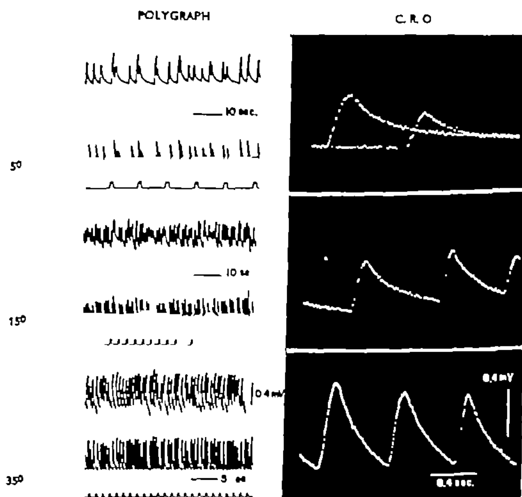


FIG. 1 Photographic samples of polygraph (*left*) and oscilloscopic (*right*) recordings of the variation of the corneoretinal potential produced by opto-kinetic nystagmus. The nystagmus was induced by rotating a drum around the cat at velocities of 5, 15 and 35°/sec. In the polygraphic records the top line is the direct nystagmus recording, the middle line the derived slow component and the bottom line the photoelectric timer.

on the forehead with acrylic cement. This platform supported three insulated stainless steel wire electrodes. The bare tips of two electrodes were placed subcutaneously at the outer canthi of the cat's eyes. For reference the third electrode was placed under the skin of the scalp. The outside tips of the recording electrodes were stapled to miniature pins. During testing the connections between the electrodes and the recording equipment were made by soldering the miniature pins to the leads of the preamplifier. The observations were started no earlier than two weeks after electrode implantation to insure adequate healing and electrode stability.

The cat was immobilized in a box similar to that described by Henrikson, Fernández & Kohut (1960). The box containing the cat was placed inside an opto-kinetic drum (a cylinder 40 inches in diameter and 30 inches in height) that could be rotated uniformly by a constant speed driving system at any desired velocity up to 120 degrees/sec. The bottom



Fig. 2. Photograph of oscilloscopic di pla f 1 nystagmic beat. The parameters ha been estimated follows. Duration of the f st component (*AD*) duration of the low component (*DC*) amplitude (*B-D*) d slope f low component *BD/DC*.

of the drum was covered with a black surface and the top by a piece of white lucite through which diffuse illumination was obtained

To produce the opto-kinetic nystagmus two different optical conditions were created In the first series of experiments the wall of the cylinder was completely black On the inner surface vertical white stripes, 5 wide were attached Two tests were made each day one using 4 stripes, and the other with 8 stripes. The tests were run in an arbitrary order

In the second series of experiments the black wall of the cylinder was removed leaving only the white stripes. The visual field of the cat was limited t a 90 arc by placing a vertical partition on each side of the animal. A black cloth was hung outside of the drum at a distance of approximately one meter thereby limiting the background space of the cat isual field Expl ratory tests were made using different numbers of stripes until it was found that 24 stripes were sufficient to produce nystagmus. All tests were performed using the same velocities (5 7.5 10 15 20 25 30 35 40 4 and 50 degrees/sec) The speed of the drum was monitored by 8 small mirrors that were equally spaced at the bottom of the drum and reflected a beam of light on a photoelectric cell the discharge of which was recorded simultaneou ly with the nystagmogram

The ariation of the corneal retinal potential produced by movements f the eyes were recorded differentially with DC coupled amplifiers (Gras 7 1 1) The amplitude of the nystagmus wa evaluated by measuring the oltage peak-to-peak produced by the nystagmic deflection. A parallel out put from this channel of the polygraph was fed into a 7P 3 Grass pre amplifier Thi preamplifier is suit ble f r measurements of the slope of the nystagmic ignals. It provided a circuit equivalent to the one used by Henrik son (1955 a) following the principles of Powsner & Lion (1950) At the beginning of each experiment the preamplifier was calibrated by mea ring the slope of the slow phase of saw-tooth pulses of known haracteri tics. From the calibration curve any slope could be calculated

For each velocity tested the amplitude of th opto-kinetic nystagmus and the lope f the slow component were obtained by averaging the v lues of ll f the nystagmic ignal recorded during a minute period The number of beat during this time interval determined the frequency (Fig 1)

In two of the cat on three different days simultaneous recordings were

made by the pen on the chart of the polygraph and were photographed from the face of the Cathode-ray Oscilloscope. By projecting the photographs of the oscillographic records enlarged traces of the nystagmus were obtained (Fig 1 right) These traces enabled more accurate measurements of the fast and slow components of nystagmus The amplitude of the nystagmus both in the polygraph and in the oscilloscopic records was determined by measuring the voltage peak to-peak of the signal The slope of the slow component was evaluated from the photographic records by determining the time required for the signal to diminish to $^{1}/_{2}$ of its total amplitude as measured from the peak of the nystagmus (Fig 2)

RESULTS

A *Frequency*

In seven cats the frequency of the opto-kinetic nystagmus produced by rotating the drum at the specified velocities using different numbers of stripes was investigated In six of the cats the difference in the frequency of the opto-kinetic nystagmus produced by having 4 or 8 stripes was studied The test results of a representative animal (cat 10) are illustrated in Fig 3 This illustration exemplifies the relationship between the velocity of the stimulus and the frequency of the nystagmus of tests made on different days It also demonstrates that at any given velocity of the opto-kinetic drum the number of white stripes 4 or 8 had no significant effect on the frequency of the nystagmus Further evidence supporting the observation was obtained by removing the white stripes and rotating the cylinder with the black wall Under these conditions, nystagmus was easily obtained having approximately the same frequency as previously recorded in the presence of the white stripes (Fig 4 top)

Conditions opposite to the previous ones were created by removing the

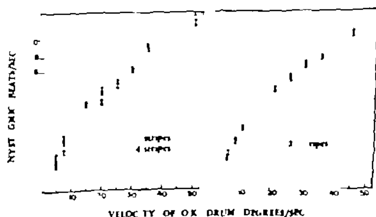


FIG. 3 Illustration of the rel tion hip between the frequency of ystagm nd the velocity f th opto-kineti stimulus ing 4 8 a d 24 stripes.

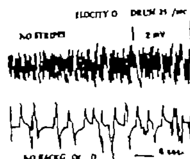


FIG. 4. Photograph of polygraph recording of optokinetic nystagmus induced by drum rotating around cat at 25°/sec. *Top*: Black drum without stripes. *Bottom*: Only white stripes were rotated, the black wall of the drum was removed.

black wall of the cylinder while leaving in place the white stripes. Under these circumstances using 8 or less stripes it was impossible to record a normal nystagmic response. Usually only a sporadic movement of the eyes was produced that was difficult to differentiate from spontaneous eye movement. In some cats after intensively arousing their attention it was

TABLE 1. *Summary of frequency measurements of each cat*

See Text. Each asterisk indicates 1 day test was not satisfactorily performed.

Cat no.	No. of stripes	Deg/sec 5	7.5	10	15	20	25	30	35	45	50
9	4	0.49	0.71	0.94	1.05	1.23	1.29	1.32	1.40	1.41	1.40
	8	0.51	0.69	0.84	1.05	1.18	1.31	1.29	1.42	1.41	1.62
	24	0.46	0.71	0.84	1.04	1.20	1.24	1.27	1.42	1.34	1.39
10	4	0.42	0.65	0.77	1.03	1.30	1.49	1.72	1.95**	2.17**	2.27*
	8	0.37	0.62	0.83	1.04	1.30	1.53	1.70	1.92*	2.19*	2.30*
	24	0.42	0.61	0.87	1.01	1.29	1.48	1.67	1.85	2.02*	1.86
14	4	0.65	0.80	1.04	1.34	1.48	1.37	1.46	1.50*	1.46	[illegible]
	8	0.72	0.65	1.03	1.38	1.62	1.63	1.64	1.51	1.42*	1.19
	24	0.48	0.73	0.92	1.17	1.43	1.60	1.67*			
6	4	0.44	0.56	0.77	1.05	1.19	1.40	1.41	1.56	1.84	1.90
	8	0.53	0.68	0.70	0.99	1.22	1.41	1.40	1.55	1.80	1.83
	24	0.53	0.70	0.80	0.96	1.01	1.14	1.14	1.41	1.38	1.55
12	4	0.52	0.75	0.88	1.06	1.23	1.26	1.42	1.47	1.59	1.71
	8	0.49	0.74	0.87	1.05	1.29	1.33	1.45	2.07	2.26	2.34
1	4	0.43	0.65	0.83	0.95	1.10	1.18	1.22	1.23	1.20*	1.33*
	8	0.49	0.68	0.83	0.96	1.11	1.23	1.32	1.25**	1.21	1.31
2	8	0.50	0.72	0.86	1.14	1.36	1.30	1.39	1.47**	1.03	1.18 **
	24	0.51	0.66	0.80	1.12	1.26	1.34	1.38	1.23	0.81**	0.82*

TABLE 2 *The mean frequencies and standard deviations for all measurements of the opto-kinetic nystagmus made at each of the velocities*

See Text.

	No. of/stripes		
Deg/sec	4	8	24
5	0.49 ± 0.07	0.52 ± 0.09	0.46 ± 0.03
7.5	0.68 ± 0.07	0.68 ± 0.03	0.68 ± 0.03
10	0.87 ± 0.09	0.86 ± 0.07	0.86 ± 0.04
15	1.06 ± 0.08	1.07 ± 0.09	1.06 ± 0.07
20	1.25 ± 0.11	1.28 ± 0.12	1.23 ± 0.13
25	1.33 ± 0.09	1.40 ± 0.12	1.36 ± 0.16
30	1.45 ± 0.18	1.44 ± 0.15	1.40 ± 0.22
35	1.52 ± 0.21	1.59 ± 0.26	1.46 ± 0.22
45	1.57 ± 0.29	1.63 ± 0.43	1.38 ± 0.42
50	1.66 ± 0.34	1.66 ± 0.46	1.40 ± 0.37

possible to produce a short sustained response with a pattern completely different from the normal (Fig 4 bottom) By increasing the number of white stripes to 24 a nystagmic reflex was easily elicited even without a rotating black wall The frequency of the nystagmus with 24 stripes was similar to that obtained in tests with 4 and 8 stripes placed on the black walled opto-kinetic drum (Fig 3 right)

Table 1 summarizes all frequency measurements For each cat the mean value of the frequency of the nystagmus produced by rotating the drum with each of the indicated number of stripes at the various velocities is shown Results for tests using 4 and 8 stripes are the mean values of the frequency measurements made on different days. The results under the heading of 24 stripes are the mean values of the measurements made on five different days

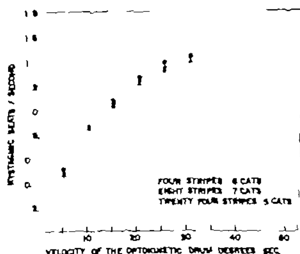


FIG. 5 Graphic illu trati of the mean l es of the freq en ies f pto-kinetic n tagmu produced t diff rent locities of opto-kinetic drum ha i g different umbers of stripes.

The mean frequencies and standard deviations for all measurements made at each of the velocities and for each of the conditions tested (4 8 and 24 stripes) are shown in Table 2. The closeness of the means and the small value of the standard deviations are proof of the significance of the tests. The mean values are graphically illustrated in Fig 5

B *Amplitude*

In each cat the amplitude of the nystagmus increased with increase in the velocity of the opto-kinetic drum. The amplitude of the nystagmus has been shown to be proportional to the angular displacement of the axes of the eyes (Fenn & Hursh, 1937 Leksell, 1939) The amplitude of the nystagmus in each cat depends among other factors upon the closeness of the electrodes in reference to the generators of the corneal-retinal potential. Since it is difficult to place the electrodes in all the animals at exactly the same place in relationship to the orbit, the magnitude of the nystagmic signal varies from animal to animal. In order to make comparable estimates in the change in amplitude of the nystagmus with change in its frequency in each cat a relative reference was taken The mean value of the amplitude of the nystagmus obtained at any velocity was measured as a proportional change in reference to the amplitude of the nystagmus obtained at 30

TABLE 3 *Normalized amplitude measurements of opto-kinetic nystagmus*

All [illegible] were in reference [illegible] the [illegible]mplitude [illegible]f the nystagmu[illegible] produced [illegible] 30°/sec. The absol[illegible] [illegible]alues of the nystagmus [illegible]re shown in the [illegible] right column. (See Text.) Each asterisk indicates 1 day [illegible] test was not satisfactorily performed.

Cat No.	No. of stripes	Deg/sec 5	7.5	10	15	20	25	30	35	45	60	Absolut value, μV (at 30)
9	4	0.49	0.57	0.59	0.74	0.86	1.13	1.00	1.13	1.06	1.09*	444
	8	0.54	0.60	0.61	0.71	0.84	0.85	1.00	1.06	1.12	1.02	472
	24	0.56	0.50	0.71	0.72	0.86	0.97	1.00	0.94	1.05	0.90*	449
1	4	0.62	0.77	0.77	0.94	0.96	0.97	1.00	0.86	1.19*	0.99*	430
	8	0.78	0.75	0.82	0.90	1.03	0.96	1.00	1.03*	1.03	0.96*	459
	24	0.65	0.74	0.84	0.89	0.91	0.94	1.00	1.07	1.16	1.15	444
	4	0.90	0.89	0.85	0.85	0.94	1.05	1.00	1.04	1.15	1.24	316
	8	0.81	0.77	0.81	0.88	0.90	0.89	1.00	1.04	1.03	1.06	366
	24	1.03	0.90	0.97	1.05	1.06	1.16	1.00	1.09	1.14	0.99	306
14	4	0.67	0.74	0.87	0.89	1.03	0.98	1.00	1.05	1.15	1.09*	254
	8	0.61	0.61	0.65	0.78	0.87	0.91	1.00	0.94	1.02*	1.04	301
11	4	0.84	0.69	0.77	1.00	0.96	1.01	1.00	1.06	1.29	1.16	337
	8	0.53	0.66	0.80	0.93	0.99	0.97	1.00	1.06	1.31	1.31	242
1	4	0.79	0.76	0.81	0.86	0.95	1.05	1.00	1.13*	1.06	1.23	261
	8	0.82	0.84	0.73	0.78	0.82	0.92	1.00	0.94	1.06*	1.06	424

TABLE 2 *The mean frequencies and standard deviations for all measurements of the opto-kinetic nystagmus made at each of the velocities*

See Text.

	No of stripes		
Deg/sec	4	8	24
5	0 49±0 07	0.52±0 09	0 48±0.03
7 5	0 68±0 07	0 68±0 03	0 68±0.03
10	0 87±0 09	0 86±0 07	0 86±0.01
15	1 06±0.08	1 07±0.09	1 06±0 07
20	1 25±0 11	1.28±0.12	1 23±0 13
25	1.33±0 09	1 40±0 12	1.36±0.16
30	1 45±0 16	1 44±0.15	1 40±0.22
35	1.52±0 21	1.59±0.26	1 48±0.22
45	1 57±0 29	1 63±0.43	1.38±0 42
50	1 66±0 34	1 66±0 46	1 40±0.37

possible to produce a short sustained response with a pattern completely different from the normal (Fig 4 bottom) By increasing the number of white stripes to 24 a nystagmic reflex was easily elicited even without a rotating black wall The frequency of the nystagmus with 24 stripes was similar to that obtained in tests with 4 and 8 stripes placed on the black walled opto-kinetic drum (Fig 3 right)

Table 1 summarizes all frequency measurements For each cat the mean value of the frequency of the nystagmus produced by rotating the drum with each of the indicated number of stripes at the various velocities is shown Results for tests using 4 and 8 stripes are the mean values of the frequency measurements made on different days The results under the heading of 24 stripes are the mean values of the measurements made on five different days

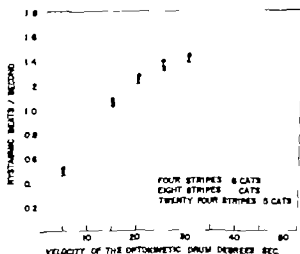


FIG 5 Graphic ill trati n f the mea val es of the frequ ies f pto-kinetic ny t gm produced t diff re t elocities f opto-kinetic drum ha i g differe t numbers f tripes.

C. *Slope of the Slow Component*

The slope of the slow component of the nystagmus, in each cat, increased with the increased velocity of the opto-kinetic drum Since the absolute amplitude of the corneal-retinal potential recorded varies for different cats, it is reflected in the magnitude of the slope of the slow component of the nystagmus. In order to compare the measurements made from the records of different cats, the data was handled in a manner similar to that of the amplitude of the nystagmus (Table 5) This data was obtained from the same tests as that of Table 3 The means of the absolute values of the slope of the slow component at the velocity of 30 degrees/sec of the opto-kinetic drum are shown in the far right column of Table 5

The normalized results from all cats of the change in the slope of the slow component with changes of the opto-kinetic stimuli are seen in Table 6 and are graphically represented in Fig 7 Since the slope of the slow component is an indication of the angular velocity of the eye in the nystagmic movement, the change of the slope of the slow component can be considered a change in the velocity of the eye. It is evident from this data that the velocity of the eye during the slow component of the opto-kinetic nystagmus increased as th velocity of the opto-kinetic stimulus was in creased. However the relationship between the change in the velocity of the opto-kinetic drum and the corresponding change in the slope of the slow component were quite different. For any increment of the velocity

TABLE 5 *Normalized measurements of the slopes of the slow components of opto-kinetic nystagmus.*

All alues were in reference t the val es of the slope of the slow component of the nystagmus produced 30°/sec. The absolute alues of the nystagmus l shown in the far right column. (See Text.) Each sterisk indicates 1 day' test was not satisfactory

Cat no.	No. of stimpts	Deg/sec 5	7.5	10	15	20	25	30	35	45	60	Absolute value, µV/sec (at 30°/sec)
9	4	0.48	0 62	0 67	0.74	0.93	0.96	1.00	1.08	1.52	1 15	1337
	8	0.41	0.45	0 47	0.66	0 78	0.88	1 00	0 99	1.09	1.02*	1465
	24	0.44	0.44	0 65	0 65	0 88	0.97	1 00	0.99	1.07	0.88	1371
10	4	0.44	0.48	0.56	0.74	0.85	0.87	1.00	1.23*	1 68	1 74	1243
	8	0 47	0.47	0 67	0.68	0.80	0.81	1 00	1.02	1.30*	1 44	1343
	24	0.44	0.51	0 66	0.69	0.78	0.87	1.00	1 17	1 40	1.36	1613
6	4	0.32	0 47	0.64	0.66	0 79	0.89	1.00	1 12	1 14	1.30	1132
	8	0 60	0.60	0.65	0.73	0 83	0.92	1 00	1 11	1.24	1.29	1111
	24	0 60	0.63	0 69	0.77	0.87	0.94	1 00	1 10	1.02	1.01	898
14	4	0 72	0 54	0.77	0.87	0.99	1.00	1.00				629
	8	0 40	0 46	0.49	0 69	0.81	0 82	1.00				802
13	4	0 32	0 44	0.54	0 75	0 87	0.96	1 00	1.23	1.66	1.60	519
	8	0.41	0.47	0.52	0 70	0.82	0 91	1.00	1.29	1 70*	1 76	487
1	4	0.36	0.54	0 66	0.76	0 86	0.99	1.00	1.12*	1 10*	1.33*	742
	8	0.44	0 44	0 53	0.60	0 70	0 82	1.00	0.94	1 10*	1.03	934

TABLE 4 *The mean and standard deviations of the amplitude measurements of the opto-kinetic nystagmus made at each of the velocities.*

Deg/sec	No of/stripes 4	8	24
5	0 67 ± 0 13	0 65 ± 0.10	0 74 ± 0.20
7.5	0 73 ± 0 09	0 67 ± 0 06	0 71 ± 0 16
10	0 77 ± 0.09	0 74 ± 0 07	0.84 ± 0.10
15	0 88 ± 0 08	0 82 ± 0 08	0.88 ± 0.13
20	0 96 ± 0 03	0 90 ± 0.07	0 95 ± 0 09
25	1 03 ± 0 05	0.91 ± 0.04	1 02 ± 0.09
30	1 0	1 0	1 0
35	1 06 ± 0 05	1 01 ± 0 05	1.01 ± 0.05
45	1 15 ± 0 07	1 09 ± 0 10	1 11 ± 0 04
50	1 13 ± 0 08	1 07 + 0.11	1 01 ± 0.10

degrees/sec The results are shown in Table 3 The values for the tests with 4 and 8 stripes were obtained from measurements made on three different days while the values with 24 stripes are the means of the measurements made on four different days The mean of the absolute values of the nystagmic amplitudes obtained with an opto-kinetic stimulus of 30 degrees/sec is shown in the far right column of Table 3 Table 4 shows the means and standard deviations of the normalized amplitude measurements from all tests in all cats These results are graphically illustrated in Fig 6

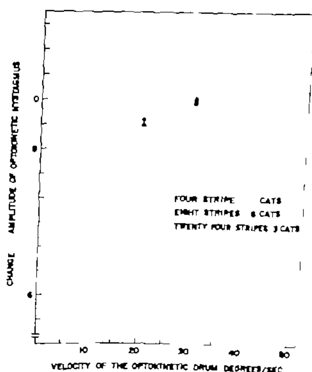


FIG 6 Graphic illustrati n of the mea val e f th amplitud f opto-kinetic ny t gmus prod ed at different elocities f the pto-kineti drum h ving different numbers of stripes.

C. *Slope of the Slow Component*

The slope of the slow component of the nystagmus, in each cat, increased with the increased velocity of the opto-kinetic drum. Since the absolute amplitude of the corneal-retinal potential recorded varies for different cats, it is reflected in the magnitude of the slope of the slow component of the nystagmus. In order to compare the measurements made from the records of different cats, the data was handled in a manner similar to that of the amplitude of the nystagmus (Table 5) This data was obtained from the same tests as that of Table 3 The means of the absolute values of the slope of the slow component at the velocity of 30 degrees/sec of the opto-kinetic drum are shown in the far right column of Table 5

The normalized results from all cats of the change in the slope of the slow component with changes of the opto-kinetic stimuli are seen in Table 6 and are graphically represented in Fig 7 Since the slope of the slow component is an indication of the angular velocity of the eye in the nystagmic movement, the change of the slope of the slow component can be considered a change in the velocity of the eye. It is evident from this data that the velocity of the eye during the slow component of the opto-kinetic nystagmus increased as the velocity of the opto-kinetic stimulus was increased. However the relationship between the change in the velocity of the opto-kinetic drum and the corresponding change in the slope of the slow component were quite different For any increment of the velocity

TABLE 5 *Normalized measurements of the slopes of the slow components of opto-kinetic nystagmus.*

All [illegible] were in reference t the [illegible] of the slope of the slow component of the nystagmus produced 30°/sec. The absolute values of the nystagmus is shown in the far right column. (See T xt.) Each [illegible] indicates 1 d y' test was not satisfactory

Cat	No. of stripes	5	7.5	10	15	20	25	30	35	45	50	Absolute value µV/sec (at 30°/sec)
9	4	0.48	0.62	0.67	0.74	0.93	0.96	1.00	1.08	1.32	1.15	1337
	8	0.41	0.45	0.47	0.66	0.78	0.84	1.00	0.99	1.09	1.02**	1465
	24	0.44	0.44	0.63	0.65	0.88	0.97	1.00	0.99	1.07	0.88	1371
14	4	0.44	0.48	0.55	0.78	0.83	0.87	1.00	1.28*	1.68*	1.74	1243
	8	0.47	0.47	0.57	0.68	0.80	0.81	1.00	1.02	1.36	1.44	1313
	24	0.44	0.51	0.66	0.69	0.78	0.87	1.00	1.17	1.40	1.38	1513
6	4	0.52	0.47	0.54	0.66	0.79	0.89	1.00	1.12	1.14	1.30	1132
	8	0.60	0.60	0.65	0.73	0.83	0.92	1.00	1.11	1.24	1.29	1111
	24	0.60	0.63	0.69	0.77	0.87	0.94	1.00	1.10	1.02	1.01	895
11	4	0.72	0.88	0.77	0.87	0.99	1.00	1.00				629
	8	0.46	0.48	0.49	0.69	0.81	0.82	1.00				802
12	4	0.32	0.41	0.54	0.73	0.87	0.96	1.00	1.23	1.08	1.60	519
	8	0.41	0.47	0.52	0.70	0.92	0.91	1.00	1.29	1.70*	1.76	487
1	4	0.56	0.61	0.66	0.78	0.88	0.99	1.00	1.12	1.10*	1.35*	742
	8	0.44	0.44	0.53	0.69	0.70	0.82	1.00	0.98*	1.10*	1.03	994

TABLE 6 *The mean and standard deviations of the measurements of the slope of the slow component of the opto-kinetic nystagmus made at each of the velocities*

Deg/sec	No. of stripes 4	No. of stripes 8	No. of stripes 24
5	0.50 ± 0 12	0 45 ± 0 06	0.49 ± 0.07
7 5	0.52 ± 0 06	0 48 ± 0 05	0 52 ± 0.07
10	0 62 ± 0 08	0.53 ± 0 05	0 66 ± 0.01
15	0 76 ± 0 06	0 69 ± 0 02	0 0 ± 0.05
20	0 88 ± 0 06	0.80 ± 0 06	0.84 ± 0 04
25	0.94 ± 0 01	0 86 ± 0 04	0 92 ± 0 04
30	1 0	1 0	1 0
35	1 16 ± 0 07	1 07 ± 0.11	1 08 ± 0 07
45	1.38 ± 0.21	1.29 ± 0.22	1 16 ± 0.18
50	1 42 ± 0._1	1 42 ± 0.25	1 08 ± 0.18

of the opto-kinetic drum the increase in the slope of the slow component was less than the stimulus increment

The absolute value of the slope of the slow component made by the electrical methods was calculated from the calibration curves of the apparatus In order to check the validity of the results, in two cats, the

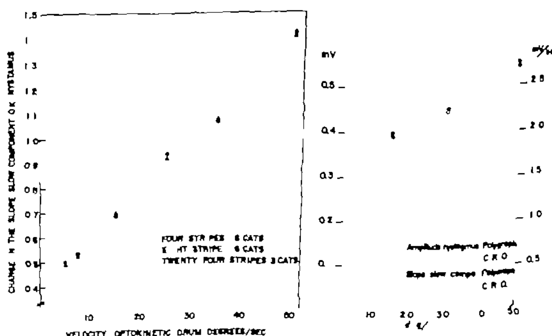


FIG 7 Graphic illustrati of the mea values of the l pe of th slow component of opto-kin ti nyst gmus prod ced t different locities f th pto-kinetic drum ha ing differe t numbers of tripes.

FIG 8 Comparison of mea urements f th mplitud nd f the sl pe of the low compon nt f the opto-kinetic nystagm mad simult neou ly n th polygraph nd on the cathod ray oscilloscope Ea h point i the mea f me rements made on three diff rent days.

TABLE 7 *Measurements of the duration of the fast and slow components of opto-kinetic nystagmus from enlarged photographic records taken from the face of the oscilloscope*

Each value is the mean of measurements made on 3 different days.

Deg/sec	Fast component (duration, msecs)		Slow component (duration, msecs)	
	Cat 9	Cat 10	Cat 9	Cat 10
5	200	150	413	529
7.5	183	148	405	484
10	178	145	377	438
15	189	133	380	395
20	167	133	322	379
25	182	132	312	373
30	161	121	310	336
35	168	118	303	308
40	166	110	303	282
45	194	111	333	266
50	194	126	373	344

nystagmic signals were photographed from the face of the oscilloscope. Manual measurements were made of the slope of the slow component from the enlarged photographic records. The mean values of the amplitude of the nystagmus and of the slope of the slow component obtained by the two different methods were remarkably close (Fig. 8)

D Duration of the Fast and Slow Components of the Opto-kinetic Nystagmus

Measurements of the duration of the fast and the slow components of the nystagmus were made from the same photographic records. The duration of the fast component corresponded to the time taken from the beginning of the beat to the point of maximum amplitude of the nystagmic signal (Fig. 2, *AD*) A straight line (*BC*) was drawn from the peak of the nystagmic signal to the baseline of the record (Fig 2, *ADC*) This line crossed the trace of the slow phase of nystagmus at a point that was one third of the amplitude of the nystagmic beat. The distance (Fig. 2, *DC*) was taken as the duration of the slow phase The values obtained for the duration of the slow component were smaller than the actual values. The error therefore becomes less significant as the frequency increased (Fig 1) From this data (Table 7 and Fig. 9) the time constant of the nystagmic signal could easily be calculated.

With increase in the frequency of the opto-kinetic nystagmus diminution of the duration of the slow and of the quick components occurs. The duration of the slow component may diminish to 50% while that of the quick component may be reduced by 15 to 25%

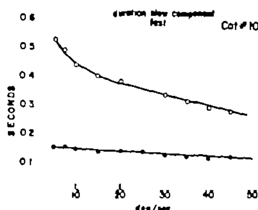


FIG. 9. Graphic representation of measurements of the duration of the fast and slow components of opto-kinetic nystagmus from Table 7 (cat 10).

DISCUSSION

The reflex mechanism responsible for the production of the opto-kinetic nystagmus showed physiological constancy especially in the middle range of the velocities tested. The measurements remained unaltered even when some of the cats were tested at intervals of up to 7 months.

Since the frequency of opto-kinetic nystagmus is independent of the number of white stripes, it is evident that the cats were moving their eyes under the influence of the rotatory stimulus but were not following the white stripes. Had the cats followed the stripes the frequency of the opto-kinetic nystagmus at the velocity of 45° per second would have been approximately 0.5 beats per second when using 4 stripes and 1 beat per second when 8 stripes were used. The data in Table 1 indicates that this was not the case. The white stripes appeared to facilitate the production of the opto-kinetic reflex. When the white stripes were removed the nystagmus was not as easily produced especially at high velocities.

Because the true nature of the nystagmus is a pursuing movement by the eye of the rotatory background, the velocity of the nystagmus should never be larger than the velocity of the stimulus. The data showed that the relationship between the velocities of the stimuli and those of the eyes was not a unitary linear relationship. The slope of the function is less than one, therefore the tests in which the velocity of the stimuli and those of the eyes must have been closest were at the lowest velocities tested (i.e. 5 degrees/sec). The difference between the velocity of the eye and that of the rotatory stimulus became larger with increased velocity of the opto-kinetic drum.

The precise relationship between the velocity of the stimulus and that of the nystagmus is unknown. To obtain this information it is necessary to know the voltage corresponding to a given angular deflection of the eye for each cat and this has not been determined.

These experiments indicate the importance of the velocity of the opto-kinetic stimulus on the production of nystagmus. It can be postulated that

in a first fixation impulse the eye tries to fix the gaze on a point within the visual field. At low velocities of the opto-kinetic stimuli the fixation of the gaze should be accomplished more easily than at higher velocities. At higher velocities the movement of the eye lags behind the velocity of the stimulus and the gap between them becomes greater as the velocity of the opto-kinetic drum is increased. The close relationship between the frequency of the nystagmus and the velocity of the *stimulus provides a* simple experimental test of the reflex mechanisms of nystagmus.

The relationship between the duration of the fast and the slow components of the nystagmic beat obtained in this study is in agreement with previous investigations (McCough & Adler 1932 Lorente de Nó, 1935 Orita, 1943) The duration of the fast component is of the same order of magnitude as the time required for the active contraction of the isolated muscles responsible for the movement of the eyes during this phase of the nystagmus (antagonist muscles, Lorente de Nó, 1935) Although the duration of the quick component varies within narrow limits it is evident that it changes with changes in the frequency of the nystagmus. The decrease in the duration of the fast component is not as large as the diminution observed for the duration of the slow component. The ratio of the duration of the slow to the fast component becomes smaller the greater the frequency f the opto-kinetic nystagmus. Increase in the frequency of nystagmus is accompanied simultaneously by increase in its amplitude and a shortening of the quick phase therefore the velocity of the eyes is increased. The behavior of the quick phase of the opto-kinetic nystagmus is imilar to the behavior of the quick phase of the vestibular nystagmus (Kolke 1959)

Measurement of the velocity of the eye duri g the opto-kinetic nystag mus in humans (Hallpike & Hood, 1953 Aschan, 1955 Henriksson, 1955 *b*) made possibl n experimental evaluation of the theory of cupula ampularis fun tion (an Egmond Groen & Jongkees, 1949) Further quantitative measurements of the opto-kinetic nystagmus in the cat will enable us to test the theory of the vestibular function in this animal

ZUSAMMENFASSUNC

Opt ki et scher Nystagmu In ormalen Katze wurde unter verschiedenen Bedingungen untersucht. Die Beziehungen zwischen den erschiedenen Parametern des N stagmus und den optokinetischen Reizen waren durch wiederholte Proben h adurch b zu Perioden n ieben Monaten beständig.

Die Häufigkeit des opt kinetischen Nystagmus war hauptsächlich von der Geschwindigk it des optokinetischen Rei es bhängig u d nabhä gig von der Zahl der an der W nd der optokinetischen Trommel befestigten Streifen Der H f gk tuumf g d die Geschwindigkeit des langsamen Besta dteil des Ny tagmus erhöhten sich mit der Erhöhung der Geschwindigk it der opt kinetischen Tromm l bis in Ma imum von 40-50 Grad Sec Reizgeschwindigkeit erreicht wurd Die Geschwindigk it des Auges während des langsamen Teils des Nystag-

mus ist nicht beständig Sie hat einen exponentiellen Lauf die Geschwindigkeit wird fortschreitend geringer während jedes nystagmischen Schlages.

Die Dauer der schnellen wie der langsamen Teile verkleinert sich mit der Erhöhung der Häufigkeit des Nystagmus. Die physiologische Bedeutung dieser Messungen wird betont

REFERENCES

ASCHAN G 19.5 The mechanism of th cupula ampullaris in ma *Act Soc Med. Upsal* *60* 3

BENDER, M B 1964 *The Oculomot r Sy tem* Harper and Row New Y rk.

TER BRAAK, J W G 1936 Unt rsuchung n über optokinetischen N stagmus *Arch. Neerl Physiol* *21* 300

CORDS, R 1926 Optisch mot risch s F ld und optisch m torische Bahn. *Arch Ophth. (Par)* *117* 58

DODGE, R and FOX, J E., 1928 Opti Ny tagmus. I Technical introduction with bservation in a case with central scotoma in the right e and external rectu palsy in th left eye *Ar h. Neurol Psychiatry* *20* 812.

EGMOND, A A J VAN, GROEN J J nd JO GKEES, L. B. W 1949: The mecha ics of the semi ircula canal *J Phy iol* *110* 1

EHLERS, H 1926: On optically elicited nyst gmus. *A ta Ophthal* (Kbh) *3*, 234

ENOKSSO P 1956 Optokinetic ny tagmus in brain le i s. *Acta Ophthal* (Kbh.) *34*, 163

FENN, W O and HURSH J B 1937 Movements of the yes when the lids are losed. *Am J Phy iol* *118* 8

GRUTTNER, R 1939: Experime telle U t rsu hung n über den pt kinetischen N stagmus *Z Sinn physiologie* *68* 1

HALLPIKE, C. S nd HOOD, J D 1953: The peed of the slow compo ent of ocular ny tagmus i duc d by a gul accel ration of th head It perime tal determination d applic ti n t the phy ical theory of the cupular mechanism *Proc. Roy Soc (B))* *141* 216

HENRIKSSON N G 1955 *a* An lectrical method f r registrati d analysi f the mo m nt f the es in nystagmus. *Acta Otolaryng* (Stockh.) *45* 25

— 1955 *b*: The correl ti n between th peed f th ey i th low phas of ystagmus nd estibular tim la *Acta Ot laryng* (Stockh) *45* 120

HENRIKSSO N G F R L CE, C., nd KOHUT R I 1960 The c loric test in the al. *A ta Otolaryng* (Stockh) *53* 21

KOIKE, Y 1959 An observati n the ye peed of n t gm s. *Acta Otol ryng* (Stockh.) *50* 377

LENNERSTRAND, L 1939 Clinic l rec rding f eye-m m t *A Ch r Scand* *82* 262

LORENTE DE NÓ, R 193. Observ ti ns yst gmu *Acta Otol ryng* (Stockh.) f 416

MACKENSEN G 1954 In tig tion f the physi logy of the opt kinetic nyst gmu I di id lly co ditio ed qua titati diff rence d i g th course f the pt kin tic ny t gmu *(a f A h. Ophthal* (Kbh) *155* 284

McCOUCH, C P nd ADLER, F H 1932 Extraocular refl es *Ame J Physiol* *100* 78.

OHTA, T 1943 Son b rv ti both th q ick d th l w compo ent in labyrinthin nyst gmu (J p.) *Jibi R nsh* *38* 3 (q oted by h lk 1939)

POWSNER, F R nd LIO K S 1950 Testi g eye muscles *Elect onics* *23* 96.

R DEM ER, G C J nd TER BRA K, J W C 1948 On th central mechani m f some ptic reacti ns. *Bra n* 1 48

D p f Ot l ryngology and th Bill Will r n H ring & Speech Cent r of V nd rbilt Univers ty S hool of Med cine Nash ill T nn U S A

R ce ed M h 4 1967

Acta oto-laryngologica 64 402–414 (1967)

GALVANIC PSEUDONYSTAGMUS

Electronystagmographically recorded Nystagmus-like Reaction induced by Galvanic Action

K. Breson and E. Krag
Copenhagen, Denmark

From the E. N. T. Department (Head: H. Rasmussen), The Finsen Institute, Copenhagen, and the E. N. T. Department (Head: C. M. Smidt), The Central Hospital, Naestved

On the basis of clinical ENG using bipolar galvanic stimulation (binauricular or monoauricular) the traced curves allow a distinct differentiation between a long and a short phase in the reaction at BB as well as at BM. The direction of the short phase altered according to the position of the cathode. It is clearly apparent also that the regular rhythm is synchronous with the cardiac rhythm in the simultaneously recorded cardiogram, only delayed in phase.

Concurrent measurements of the decrease in voltage between the lead-off electrodes during stimulation showed that the interference voltage is, on average, 800 (1100–600) times higher than the recorded voltage variations. It is also apparent that the monoauricular stimulation predominantly affects only the stimulated side. On the basis of the literature and the curves submitted in the present paper it must be concluded that direct ENG is not applicable as a recording method in the galvanic test, as the tracings merely represent resistance variations between the lead-off electrodes synchronous with the heart beat.

Attempts have been made to systematize for clinical use the finding that galvanic action upon the vestibular apparatus elicits not only dizziness, vertigo and deviation of the head, but also jerky movements of the eyes (Buys, 1909; Mackenzie, 1917; Christiansen, 1958). At direct currents from mA to 20 mA nystagmus can be observed either behind Bartels' goggles or direct.

However, then just as *now* the recording was the main problem. Buys (1909) used kymatography, Dohlman (1929) photoelectronystagmography, Jongkees & Philipszoon (1964) electronystagmography by the method of Aschan, Bergstedt & Stahle (19 7) and Richter & Pfaltz (1958) and Gabersek & J bert (1963) also used photoelectronystagmography.

Jongkees & Philipszoon (1964) pointed out that ocular movements re-

Electrical engineer, Reader at the Technical Academy, Copenhagen.

corded electronystagmographically during direct current stimulation differed from nystagmus induced by other means They found no rapid and slow phase On the other hand Richter & Pfaltz (1958) using bipolar binauricular stimulation characterized the galvanic reaction as having a distinct phase difference, that is when traced photoelectronystagmographically Direct electronystagmography they found to be inapplicable because of electrical artefacts.

Gabersek & Jobert in 1965 demonstrated that the regular rhythm recorded on an electronystagmogram is synchronous with the cardiac rhythm and essentially different from photoelectronystagmographic results.

The present paper reports (*a*) a technique of galvanic stimulation, recording by electronystagmography and the results obtained in clinical experiments, and (*b*) measurements of the magnitude of interference phenomena caused by the stimulation current during the recording

TECHNIQUE

The stimulation was induced in both experimental series, by a 12 V battery in series connection with a linear variable resistance and a microammeter which may be graded from 0 to 3000 μA in 50 μA steps, i.e. a floating stimulation (Fig 1)

Silver-chlorated electrodes[1] wrapped in chamois are mounted on the spring band of earphones The electrode arrangement permits bipolar binauricular or monoauricular stimulation

In the bipolar binauricular (BB) arrangement the electrodes may be placed on the mastoid process or on the tragus (Fig 3)

In the bipolar monoauricular (BM) arrangement the electrodes may be placed around the auricle one on the mastoid process and the other one on or just anterior to the tragus (Figs. 2 and 3)

The skin is washed with ether at the sites of stimulation and at the leading-off sites at the lateral canthus of the eyes Only a horizontal lead-off was used The chamois around the silver electrodes is soaked with physiological saline Moreover the leading-off electrodes are smeared with EEG electrode paste

The curves were recorded by an Elema Schönander Cardirex mingograph 31 B with a transistorized pre-amplifier EM 12 from the same makers. In all the clinical experiments the sensitivity is adjusted to 100 μV/cm the upper 3dB frequency is 15 cps, and the time constant 2 5 sec.

Thus the nystagmographic set up according to Aschan Bergstedt & Stahle (1957) does not differ from the ENG which now is in common use in most Danish E.N T departments too

[1] Kindly mad by Bent Gabri lsen Peders n, g ld mith, K ldi g, De ma k.



Fig. 1. The stimulatio ggregat



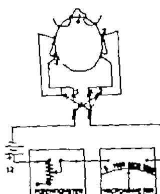


Fig. 2. EM lectrode placement on the right id Lead-off lectrode t the external canthus of the ey Indifferent lectrode on the forehead.

Fig. 3. Methods of stimulation nd directio *of the licited reaction when the electrodes* re placed as indicated.

PROCEDURES

(a) *The Clinical Experiments*

(1) The taking of the history and the ordinary objective oto-rhino-laryngological investigation were supplemented by a neurological examination

(2) Thereafter an ordinary ENG was done with a view to spontaneous or positional nystagmus, and the Hallpike differential caloric test was performed

The measuring unit was calibrated so that an ocular deviation of 10

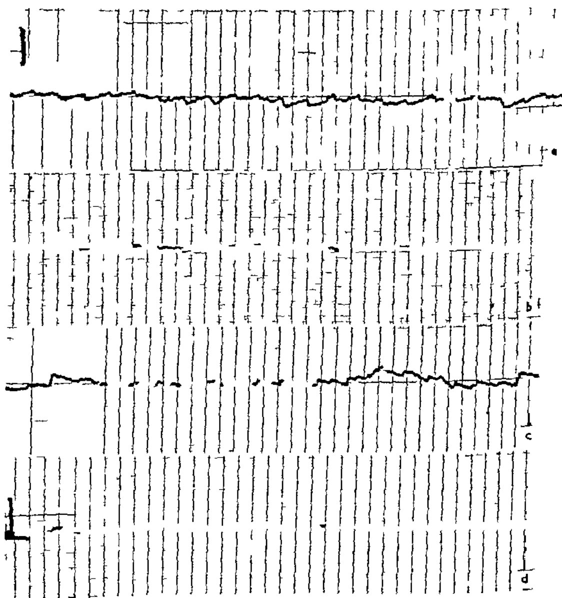


Fig 4 BB stimulati n: (Cathod l ft tragu 1300 μA 100 \ m 10 m R c-tio with h rt ph se d w w rd l "t th l ft (b) C thod o l ft tragu 700 \ 100 μV/cm. 10 /cm Reacti n barel read bl ( ) Cathod o right tragu 1500 μA 100 μV/ m. 10 /cm. Reacti n with l ort ph se upw rd i.e "t th right (d) Cathod on ight tragu 1500 / A 100 μV m 10 m. R cti t re d bl

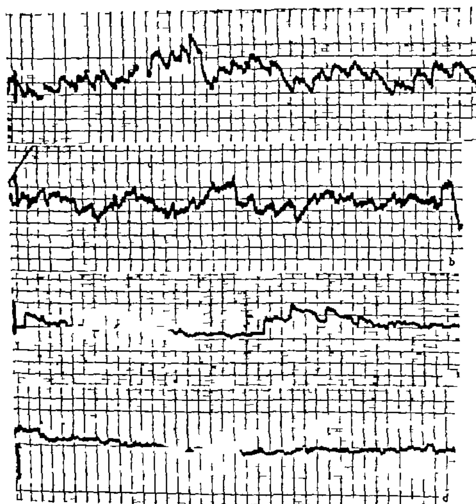


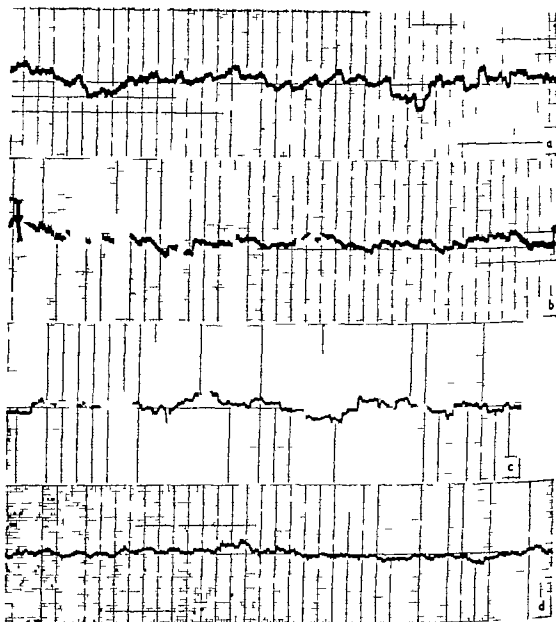
Fig. 5. BM at mulation, left ears ( ) Cathode on left mast. proces 1500 μA. 100 μV cm Rapid phase pw rds, l "t the right w y from the cathode (b Cathode on left tragu 1,00 A, 100 V cm. 5 cm Rapid phase downw rds, i.e. "t the left towards he cathode At and b) there is di t nct phase shift The nlogograph mplify g were sed. I nd d) the same electrode placement nd mpl fyi g in ( ) and b but only 700 A Reaction less distinct and not readabl

correspond to 10 mm deflection on the recorder The patient lay with closed eyes n a non-darkened room

By (1 nd (2) we mad ure that only normal persons were included After bri f pause w continued, performing stimulation by a galv nic current follow

IIII, electrodes on the tragus, with the cathode first on the left nd then n the right side

The microammeter w adjusted t 1 00 μA, the pre-amplifier wa



F o 6 BM timulati n, right ear: (*a*) Cathod on ight ma t id process, 1500 μA, 100 μA m 20 /cm R p d phase "t the l ft aw y from the ea (*b*) Cathod the right tragu 1500 μA 100 μA m 20 cm Rapid phase w "t the right ( ) and (*d*) 700 μA ight m stoid p occ and right tragus. Th reacti m y be read, also it reversib l ty but it l n t di tinct

blocked for a short time to avoid overloading and a curve was traced for about half a minute Thereafter we continued in 100 μA steps down toward a presumed threshold value all segments of the curve being approximately $^1/_2$ min

The most distinct "nystagmogram" was obtained with the cathode and the anode on the tragus Both sides were tested in this way

(*b*) BM The stimulating electrodes are placed on the mastoid process and the tragus as described In the first test the mastoid process is the

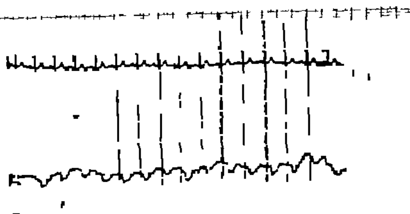


Fig. 7 Simultaneous ECG and ENG Stimulatio BB with cathode on the left tragus. 1000 μA. 100 μV/cm. 10ⁿ cm. R t 10 mm/sec. Cardiac rhythm normal ENG hythm regular synchronous with the heart beat.

cathode, in the second test the polarity is reversed that is the electrodes proper are not moved as also in BB stimulation.

Both ears are tested, starting—as with BB—the stimulation at 1500 μA decreasing by 100 μA steps, until the threshold value of the reaction has been found.

Results of the Clinical Experiments

All the normal subjects, a total of exhibited the following common features

In BB (Fig 4) all showed a recordable nystagmoid reaction at 1500 μA It is hardly possible to record a lower limit It may be stated that the reaction decreases in amplitude at decreasing current At the lower level it cannot be read, as muscular unrest blurs the result

If a threshold val e f r the lowest current which elicits a readable reaction is t be fixed on the basis of the present experiments, the alues range from about 200 to about 700 μA This is a proximately the same for both ears of each subject.

The curves show a fairly distinct division int phases, the rapid phase being toward the cathod

In BM timulation (Figs 5 and 6) the threshold values show a range fr m about 400 to about 1400 μA with all f ur ways of placing the cathode

But in addition, this way of pl cing the lectrodes shows on the curves that the rapid phase f the reaction m y be reversed. With the cathode on the ma toid process the "nystagmus" will turn away from the stimulated ear In reverse it will be towards the stimulated ear when the cathode is placed on the tragus, or immediately anterior to it

It is a main rule that in these normal subjects the reversibility of the ocular movements" could be reproduced as shown in Fig 3

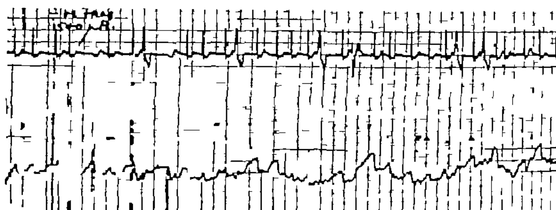


Fig 8 Simultane us ECG and ENG in a pati nt with auricula fibrillation a d extra ystoles. BB with cathod on l ft tragus, 1500 μA 100 μV/cm 10 /cm. Rat s in Fig. 7 It may be seen that the pulseless extrasystoles do not licit ENG deflecti n

Furthermore owing to the regularity of the nystagmoid reaction seen on all curves, we supplemented the experiments by simultaneous recording of the ECG in two patients

One (Fig 7) had a normal cardiac rhythm the other auricular fibrillation (Fig 8)

On the curves it may be seen that the reaction induced by galvanic action is synchronous with the cardiac rhythm only the phases are delayed 0 1–0 2 sec.

PROCEDURES

(b) The Potentiometric Measurement of Potential Distribution during Stimulation

Concurrently with the named clinical experiments we have, as mentioned measured the magnitude of the interference phenomena caused by stimulation current by measuring the distribution of potentials as a function of the lead-off site around the head

Fig 9 illustrates the experimental set up before, during and after the stimulation, which was performed as previously described for the clinical experiments On the figure the stimulation electrodes are shown as S_1 S_2, and S_3 Six lead-off electrodes marked A_1 A_2 A_6 are placed around the head from the left tragus to the right mastoid process.

The potentiometric measurement of voltage is done between the leading off electrodes A_i ($i = 2$ 6) and the reference electrode A_1 in front of the left tragus (close to the stimulation electrode S_1)

The lead-off electrode is connected to the potentiometer set up by the rotary switch O_2.

As a potentiometer we used a 10 winding Helical potentiometer marked Pot with a resistance R = 100 kohm supplied by a 6 V dry battery in series connection with a serial resistor R_s, a mV meter V and a zero galvanometer

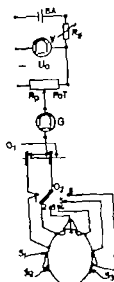


FIG. 9. Voltage measurement (cf. the text)

G with maximum sensitivity at full scale deflection ± 1 µA. The switch O makes possible the measurement of positive as well as negative voltages.

The voltage U across the potentiometer is adjusted to 100 mV by the resistor R_v. The lead-off electrodes are chosen by switch O_2, and the galvanometer is nulled by adjusting the potentiometer to ratio a by the sign switch O either in position + or −.

If a nulled galvanometer circuit is obtained with O in + position, the voltage difference between the lead-off electrodes A and the reference electrode A_u $U = aU$; thus, with O in the − position, it is $U = aU$ ($U_0 -$ U).

Results of Potential Measurements

The measurements showed that even without stimulation the lead-off electrodes have different potentials (varying electrolyte concentration beneath the electrodes?). A voltage of 20 mV magnitude was measured.

During the stimulation we ascertained changes approximately proportional to the stimulation current, but after stimulation the potentials of the lead-off electrodes altered unilaterally on an average 8 mV from their initial values.

It thus seems to have been established that the potential difference between the right and the left lateral canthus of the eyes changes about 37 mV on binauricular stimulation and only about 15 mV in monauricular stimulation.

The difference in potential between leads A and A is distinctly greater with BB stimulation, approximately 40 mV, with BM stimulation only

about 5 mV From this result it may be assumed that the BM stimulation may be rendered practically unilateral

This and the fact that there is an approximately 2?–15 mV voltage between the external canthi of the eyes are the most important findings. In other words a voltage about 1100–600 times higher than the variations in voltages recorded in an ENG set up

DISCUSSION

After Dohlman's studies around 1930 on galvanic vestibular tests, only a few workers have investigated the subject

Similar to the primary intention of the present study Pfaltz & Richter (1965) tried to develop a clinically applicable method with electronystagmographic recording However as already stated they believed that the stimulation influenced the lead-off electrodes. What they traced represented electrical artefacts

In this country Christiansen (1958) using the same technique of stimulation tried to assess galvanic electronystagmograms on a series of patients without arriving at a significant result

As also mentioned in the introduction Gabersek & Jobert (1965) demonstrated that the frequency of the nystagmoid reaction is synchronous with the cardiac rhythm, but phase-shifted like the curves in our study This indicates that what is recorded is something else than vestibularly elicited nystagmus.

These authors stimulated with more than 1000 μA and the recorded "ocular movements" directed their short phase towards the cathode at a rate of 1–2 per sec

The amplitude increases with increasing current but not in a typical linear way

In simultaneous photoelectronystagmography Gabersek & Jobert (1965) demonstrated that in the early part of the stimulation the eyeballs do not move although an ordinary nystagmogram" can be traced immediately They believe therefore like Richter & Pfaltz (1958) that the method using direct nystagmography does not record nystagmus elicited by galvanic action but that what is traced is a rheogram, a measurement of a pulse conditioned alteration of the decrease in voltage between the lead-off electrodes during stimulation

This hypothesis is supported by the finding that the direction of the rapid phase may be altered by reversion of the current of the stimulation

In the present study the stimulation was brought to bear around the vestibular apparatus, bilaterally and unilaterally whereas Gabersek & Jobert placed their electrodes in a number of different positions on the head

However in a cutaneous stimulation like this one must realize the course of the current in the integument and also study the interference phenom

em in floating stimulation (i.e. stimulation without an earth connection) when the lead is direct from the stimulated object during stimulation, as we have done in the above mentioned compensation experiments.

As stated by both Frenzel and Richter there is a question of very coarse interference in relation to voltage variations to be recorded. We found an average of 850 times higher.

During the stimulation in the first-mentioned "clinical" experiments, therefore the pre-amplifier had to be blocked simultaneously with the stimulation in order to avoid overloading which will last 5–10 sec after the stimulus.

In his paper on stimulus interference phenomena in biological specimens Guld (1960) mentioned the extremely complex factors which have to be taken into consideration in electrical stimulation, thus confirming our findings. A suitable choice of electrode distance, electrode impedance, stimulus output impedance, and pre-amplifier input impedance are of decisive importance in limiting interference phenomena in electrical stimulation and recording by direct lead from the stimulated object.

CONCLUSION

In contrast to the findings of Jongkees & Philipszoon (1964) it was possible in the present study using classical ENG to make fairly clear distinction between the slow and rapid phase of the reaction induced by galvanic action.

The electrical artefact mentioned by Richter & Pfaltz (1958) was observed also with the present method. We compensated for these artefacts in the pergalvanic curves by a short-lasting blocking of the pre-amplifier simultaneously with the stimulus, since we were primarily interested only in ascertaining a threshold value at the named positions of the electrodes.

However it must be assumed, as did Gabersek & Jobert (1963) that this method of recording does not trace a vestibular nystagmus as the curves distinctly show a synchronism with the cardiac rhythm.

The explanation of the recorded nystagmus-like reactions, synchronous with the pulse is presumably that the resistance in the tissue between the lead-off electrodes varies with and dependent on variations in the arterial blood pressure.

The attempted clinical method is therefore hardly applicable for diagnostic purposes in otoneurological investigation.

ZUSAMMENFASSUNG

Auf Grund einer klinischen Elektro-Nystagmusregistrierung bei Verwendung einer bipolaren, galvanischen Reizung (beider Ohren oder eines Ohres) gestatten die aufgezeichneten Kurven eine scharfe Differenzierung zwischen der langen

und einer kurzen Phase in der Reaktion sowohl bei BB als auch bei BM. Die Richtung der kurzen Phase wird gemäss der Stellung der Kathode geändert. Es ist auch völlig klar dass der reguläre Rhythmus — nur in der Phase verzögert — mit dem Herzschlagrhythmus in dem gleichzeitig registrierten Kardiogramm synchron verläuft

Nebenher verlaufende Messungen der Spannungsabnahme zwischen den Eingangselektroden während der Reizung zeigten dass die Interferenzspannung um ein Mittel von 850 (1100-600) mal grösser ist als die registrierten Spannungsschwankungen Es ist auch klar dass die Reizung eines Ohres vorzugsweise nur die gereizte Seite beeinflusst An Hand der Literatur und den in dieser Abhandlung vorgelegten Kurven muss die Schlussfolgerung gezogen werden, dass eine direkte Elektro-Nystagmusregistrierung als Registrierverfahren in der galvanischen Untersuchung nicht brauchbar ist da die Spuren nur die zwischen den Eingangselektroden synchron zur Herzschlagfolge verlaufenden Spannungsschwankungen darstellen

REFERENCES

Aschan G Bergstedt M and Stahle, J 1957: Electronystagmography *Acta Otolaryng* (Stockh) Suppl *129*

Buys, 1909: B itrag zum Studium des Galvanischen Nystagmus mit Hilfe der Nystagmographie *Mschr Ohrenheilk* 43 11 801

Christiansen E., 1958 Personal communication.

Dohlman G 1929: Experimentelle Unt rsuchung n übe die galvanische Vestibularisreaktion *A ta Otolaryng* (Stockh.) Suppl *8*

— 1957: *L épreuve Galvanique* p. 315 Presse Uni ersitaire de France

Gabersek V and Jobert F 1965 L épreuve Galvanique en électronystagmographie *Rev Neurol* (Par) *112* 266.

Guld, Chr., 1960: The reduction of stimuln interference i electrophysiology *Proceedings f third int co g on med électr Paris* p. 14

Jongkees, L., and Philipszoo A., 1964: Electronystagmography *Acta Otol ryng* (Stockh) Suppl *189*

Mackenzie, G 1917: Galvanic test f the i t rnal ea *Laryngoscope* 27 200

Pfaltz, C., nd Richter, H., 1965 La leur di gnostique de la réacti n vestibulaire galvanique. *Confin Neurol* 23 203.

Richter, H nd Pf ltz, C., 1958 Recherches ny t gm graphiques ur la réaction vestibulaire g lva iqu *Confin Neurol* *18* 370

K Breson M.D
Poppelbakken
Birk roed
Denmark

Received F b uary 8 1967

Acta oto-laryngologica 64, 115-126 (1967)

EFFECT OF ETHYLALCOHOL AND PENTOBARBITAL SODIUM ON THE ACOUSTIC MIDDLE EAR REFLEX IN MAN

E. Borg and A. R. Møller
Stockholm Sweden

From The Department of Physiology Karolinska Institutet Stockholm 60

Th effect of ethylalcohol d pentobarbital sodium n the coustic middle ear reflex in man wa studied by recording the change in the ears coustic impedance before and after oral dministration of ethylalcohol, giving blood lcohol concentrations from 0.02 to 0.15% and pentobarbital in doses from 1.5 to 4.5 mg kg. The reflex was elicited by burst of pure tones (500 and 1450 cps) and the response wa recorded in both ears simultaneously when ither th right r the left ear was stimulated. Thus, the effect on the crossed and the uncrossed refle es was studied

It was found that both ethanol and pentobarbital have a depressi e effect on the reflexes. The crossed d th uncrossed reflexes were equally sensitive t ethanol while the crossed reflex wa more sen itive t pent barbital than the uncrossed. In addition, the slope of the stimulus-response urve f the crossed reflex was diminished by pentobarbital, while ethanol did not hange the slope f th crossed and uncrossed refle es. The results re discussed in relation to studies on the sensiti ity f spi l refle es to ethanol and barbiturat Th consequences of dimi ished middle ear muscle activity caused by th drugs for sound-i duced d mages n the inner ea are empha ized.

In animal experiments it has been shown that both ethanol and barbiturates have a depressive effect on spinal cord reflexes (see e.g Wikler 194 Petersén, 1032 Kolmodin, 1933 Sigg, Holland & Schneider 1958 Wilkin, Spitaletta & Plummer 1960) the polysynaptic reflexes being more sen itive than the monosynaptic Some authors also report a slight aug mentation of polysynaptic reflexes at low doses of barbiturates (see, e.g Heinbecker & Bartley 1940 Chin & Smith, 1962) A depressive effect of barbiturates on cerebral reflexes has also been reported (e.g., Hoffmeister 1962 Mitchell, 1964) These studies were made on the lingulom ndibular lick ng and jaw opening reflexes.

The sensiti ity of the acou tic middle ear reflex to anesthetics has been discu sed b Wersäll (1958) who compared the maximal tension of the two middle ea muscles e ked by sound stimuli in rabbit under light pentobarbital anesthesia and in non-anesthetized, decorticated animals. He found that the mean of the maximal ten ion of the stapediu muscle in decorticated

animals was twice that in animals under light anesthesia, whereas the corresponding difference for the tensor tympani was much smaller Further surgical anesthesia has been reported to have the same effect on the cochlear microphonics as a transection of the tendons of the middle ear muscles (see e.g., Lawrence 1960)

In man no quantitative studies on the effect of alcohol and barbiturates on the middle ear reflex have been performed

The acoustic middle ear reflex can be elicited ipsilaterally as well as contralaterally the ipsilateral reflex being more excitable than the contralateral (Møller 1961) A contraction of one or both middle ear muscles changes the mechanical properties of the middle ear which can be measured as a change in the acoustic impedance at the eardrum Thus, a graded measure of the mechanical effect of the muscle contraction can be obtained. This method has been used in numerous studies of the function of the acoustic middle ear reflex (Metz, 1951 Jepsen, 1955 Klockhoff 1961 Møller 1958 1961 1962 *a* *b* 1964)

The aim of the present study is to show how the stimulus-response pattern of the human middle ear reflex changes after administration of different doses of alcohol and pentobarbital sodium (Nembutal Abbott®) The reflex response was measured by recording the change in the acoustic impedance of the ear

METHODS

The subjects were healthy and had normal pure tone audiograms. None of them had had any ear affections or neurological diseases. Their age ranged from 21 to 24 years. The acoustic impedance change was measured in both ears simultaneously using the same method and set up as has been described earlier (Møller 1961 1962 *b*) Therefore only a brief description will be given here

The impedance measuring device was attached to a tube which was sealed in the ear canal by a dental molding substance (Lastic 55) This tube was left in the ear canal throughout the experiment ensuring constant measuring conditions The impedance measuring device was in place only when measurements were performed The middle ear reflex was elicited by pure tones with frequencies of 500 or 1450 cps and a duration of 500 or 1000 msec The stimuli were delivered by an earphone inside the impedance measuring device The sound pressure level of the stimulus was varied in steps of 2 dB or 4 dB After application of the impedance measuring device to the ear the stimulus sound pressure level (dB re 0 0002 μb) near the eardrum was measured with a calibrated probe microphone in the impedance measuring device This measurement was usually made both before and after completion of the experiment

The rise time (to 90% of full amplitude) and decay time (for 90% decay) of the stimulus were both 2 ms The change in acoustic impedance

was measured at a frequency of 800 cps (continuous tone) This tone had a level near the eardrum of approximately 65 dB SPL which is well below that required to elicit the acoustic reflex (Møller 1962 a) The 800 cps tone was electrically balanced out and the change in the acoustic impedance ppeared as an 800 cps signal the amplitude of which was proportional to the magnitude of the changes in the ear s acoustic impedance The output of the impedance measuring device was recorded on magnetic tape and processed at a later date.

The stimuli were presented to the ears one after another beginning at an intensity below the threshold of the acoustic reflex The level was raised in steps until the highest level tested was reached. The level was then decreased in steps until below the threshold of contraction In that way the various stimuli intensities were tested twice. The mean value of two recordings f the impedance change measured immediately before the end of the stimulating ton was used to express the magnitude of the impedance change t a certain stimulus level The impedance change was expressed in per cent of maximal effect obtained and plotted as a function of the sound pressure level of the stimulus.

The reflex response was first recorded immediately before administration of ethanol or pentobarbital The effect of varying doses of pentobarbital gi en orally was tested 40–60 minutes after administration, i.e when the effect is maximal (see Fig. 7) The ethanol was also given orally (40% solution) and the effect was tested at various intervals after administration, but not earlier than 15 minutes after the alcohol intake The blood alcohol concentration was determined from 3 samples taken in immediate connec tion with the measurements. All experiments were made about six hours after the subject s l st previous meal

RESULTS

a Ethanol

The effect of ethanol on the acoustic middle ear reflex was studied in 9 ubject In a t tal of 11 experiments. The changes in acoustic impedance f both ears wer recorded imultaneously In ll experiments the reflex was *elicited by sound stimulation* of both ears, one at a time Fig. 1 il lustrates a typical experiment in which the change in acou tic impedance f r l all ears was recorded simultaneously while a pure tone of various ntensity wa presented The frequency of the sound was 500 cps and the durati n w 00 msec The curve showing th impedance ch nge in the c ntralateral ear is interrupted. When th magnetic tape recordings were played back th utput f the two channels, representing the impedance hange in the two ears, were ligned with respect to amplitude in such a w y that the maximal impedance hange obtained gave the sam deflections

The ADH-method (lcoholic dehydrogenase) w sed nd the nalysis was made t the Governmental Laboratory for Forensic Chemistry (Head Prof R. Ben ickse)

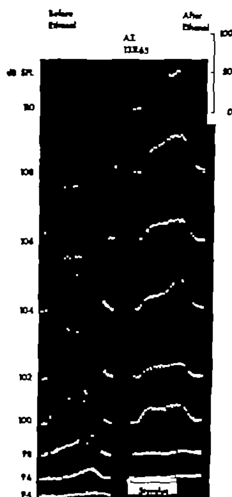


FIG. 1 Ipsilateral (solid line) and contralateral changes (broken line) in acoustic impedance at various stimulus intensities as a function of time recorded before and 30 minutes after ingestion of 105 ml ethanol when the blood alcohol concentration was 0.12%. Stimulation with a 500 cps tone with duration of 500 ms. The scale in the upper right corner gives the impedance change in per cent of the maximal obtainable change

for the two ears. The left column shows recordings immediately before administration of alcohol and the right column recordings 30 min after administration of 105 ml pure ethanol (in 40% solution). The blood alcohol concentration at the time of recording was 0.12%.

Ethanol is followed by a reduction of the middle ear reflex as shown by the diminished responses to the same stimulus intensity (to the right in Fig. 1).

Fig. 2 shows the magnitude of the acoustic impedance changes at various stimulus intensities before (solid curve) and after alcohol (dashed curve) plotted as a function of the sound pressure level of the stimulus (dB re 0.0002 μb). The frequencies of the pure tones used for stimulation were 500 cps and 1450 cps respectively and the duration was 500 ms. The change in acoustic impedance is expressed in percentage of the maximal impedance change obtained. As seen in Fig. 2 the curve is shifted to the right after alcohol administration, which implies that a higher sound

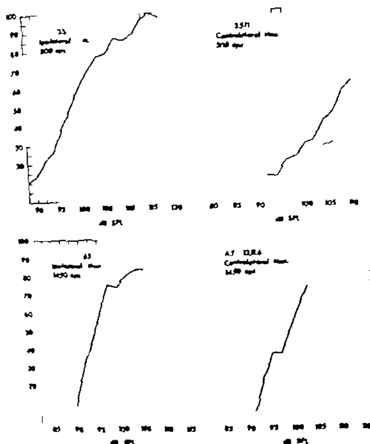


Fig. 2. Impedance change as function of stimulus intensity before intoxication and at blood alcohol concentration of 0.12% (upper diagrams) and 0.11 (lower diagrams) measured at 500 cps and 1450 cps respectively. 100% denotes maximal obtained impedance change. Each line represents the mean of two determinations.

pressure is needed in order to obtain the same impedance change as before the alcohol intake. The crossed and the uncrossed reflexes do not seem to differ significantly in their susceptibility to alcohol. Nor does there seem to be any difference in the susceptibility of the reflexes when elicited by a pure tone of 500 cps or of 1450 cps.

Fig. 3 illustrates the effect of alcohol on the ipsilateral and contralateral acoustic middle ear reflexes as a function of blood alcohol concentration. The diagram is based on 22 tests on 9 subjects and the graph shows how much the stimulus sound pressure level had to be increased after alcohol administration in order to elicit the same impedance change as before alcohol. The results in Fig. 3 were derived from curves similar to those presented in Fig. 2. The difference in sound intensity required to produce 10, 20 and 40 % of the maximal obtainable impedance change before and after alcohol was determined. The mean of three such determinations was

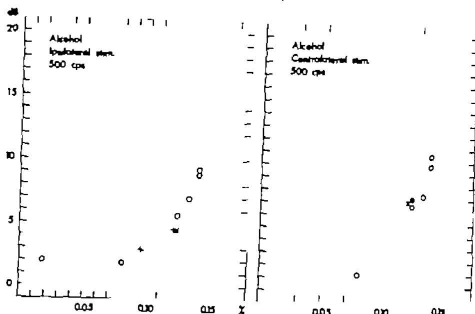


Fig 3 Incre se in stimulus int nsity necessary to obtain an unchanged refle response t ipsilateral (left graph) and contralateral (right graph) stimulation a a function of blood alcohol concentration mea ured in 9 subject The symbols represe t v lues bt ined on diff rent subjects.

used to represent the change in excitability (Fig 3) It should be mentioned that some of the results shown in Fig 3 were obtained during the same experimental session but at different times after the alcohol administration Some of the subjects were used at more than one occasion with several days interval

The mean change in excitability is plotted in Fig 4 The values for the blood alcohol concentrations were divided into four classes, and the mean values of each of these classes are shown The open circles represent the

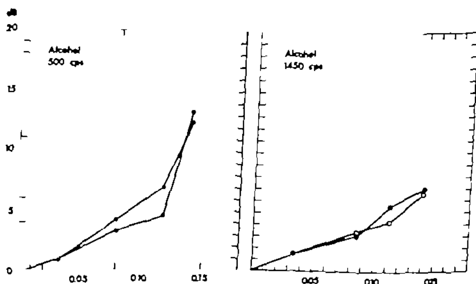


Fig. 4 Mean values f the increase in timul s i te ity necessary t obtai nd unchanged reflex response a a fu ction of blood alcohol concentrati n. Open ircles represent the uncrossed and filled ircle the crossed reflex.



Fig. 5 Ipsilateral (solid line) and contralateral changes (broken line) in acoustic impedance at various stimulus intensities as a function of time measured before and 90 minutes after oral administration of 250 mg Nembutal (3.0 mg/kg). The stimulus was 500 cps tone with a duration of 1000 ms.

ipsilateral and the filled circles the contralateral reflex. At 500 cps the stimulus intensity must be increased about 10 dB to compensate the effect of a blood alcohol concentration of 0.13%, the necessary increase being equal for the crossed and uncrossed reflexes. At a blood concentration of 0.05% ethanol the corresponding increase is about 2 dB. As shown by the diagram on the right of Fig. 4 the reflexes appear to be less susceptible when elicited at 1450 cps than at 500 cps. This difference is, however, more apparent than real since those subjects tested at 1450 cps happened to be those whose reflexes were least susceptible also at 500 cps.

As seen in Figs. 2 and 4 the ipsilateral and the contralateral reflexes seem to be equally susceptible to ethanol, although occasionally the contralateral reflex is slightly more depressed.

b. Nembutal

The effect of pentobarbital sodium (Nembutal Abbott®) on the acoustic middle ear reflex was investigated on 6 subjects in a total number of 7

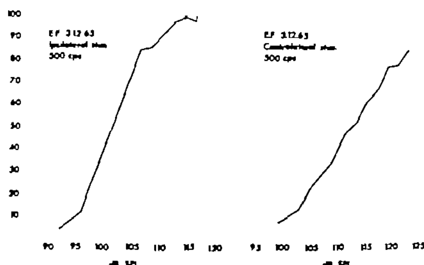


FIG. 6 Impedance change s a function of timulus intensity measured before (solid line) nd 50 min after (broken li e) administrati n of 200 mg Nembutal (4.3 mg/kg) The stimulus was a pure t ne with a frequency of 500 cps and a durati n of 500 msec presented t one of the ears. Each value represent the mean of two d terminations. The imped nce change is expressed in per cent of maximal obtainable impedance change

sessions. Measurements of the acoustic impedance change were made on both ears simultaneously when either right or left ear was stimulated with 500 msec or 1000 msec duration pure tones

Fig 5 shows the acoustic impedance change elicited by pure tone stimulation (500 cps duration 1000 msec) of various intensities before and 50 min after oral administration of 3 0 mg/kg Nembutal The crossed reflex is represented by the hatched line As seen, the administration of Nembutal is followed by a decrease of the amplitude of the impedance change and a rise of the threshold Since the shape of the contraction curves of equal amplitude is almost identical before and after administration of the drug pentobarbital in these doses does not seem to affect the temporal pattern of the reflex function In Fig 6 the amplitude of the impedance change before (solid line) and after 4 3 mg/kg Nembutal (hatched line) is plotted as a function of stimulus intensity for the ipsilateral and contralateral reflex in the same manner as in Fig 2 The reflexes were elicited by a pure tone with a frequency of 500 cps and a duration of 500 msec

It is seen from Fig 6 that the stimulus-response curve is shifted to the right after Nembutal administration showing a decrease in excitability of the reflex Furthermore it is seen that the contralateral reflex is more susceptible to the drug than the ipsilateral reflex It also appears from Fig 6 that the slope of the stimulus response curves become smaller after Nembutal this change is more pronounced for the contralateral reflex Such a change in slope is not seen after ethanol nor are there any differences in susceptibility between the crossed and uncrossed reflex (cf Fig 2)

Fig 7 shows the decrease in excitability of the ipsilateral (open circles)

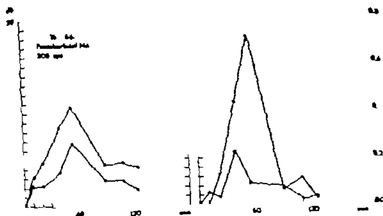


Fig. 7 The decrease i xcitability f the ipsilateral (pen circles) nd contralateral (f lled circles) middl ea refle es as function of time afte oral administration of 200 mg Nembutal (2.1 mg/kg body weight) The diagram t the left shows the decrease i xcitability of the reflex nd the right graph show th decrease i l pe of the stim lus-response curve n dB per percent impedance change The stim lus wa pure tone with frequency of 500 cps and duration f 1000 msec. Each point represent the average lue from stim lation the two ears f ne subject

and contralateral (filled circles) reflexes to a single dose of pentobarbital sodium (200 mg or 2.1 mg/kg body weight) during the two first hours after administration The average values from bilateral measurements on one subject in one experiment are shown (during alternating stimulation of the two ears) In the diagram on the left in Fig 7 the change in excit ability is expressed as the mean of the increase in sound intensity necessary to produce an impedance change equal to 10 20 and 50% of the maximal change obtained before intake of the drug (left graph) As seen the maximal effect appears after about 50 minutes and there is still a significant effect two hours after the administration. This diagram also indicates that the contral teral reflex is more sensitive to Nembutal than the ipsi lateral ne (cf Fig 6)

Since the lope of the stimulus response curve changes after Nembutal (see Fig. 6) the effect f the drug on the properties of the reflex cannot be fully described by the difference in excitability determind at a few points of the timulus-response curve The graph on the right in Fig 7 shows the decrease n slope of the stimulus response curves. The slope was measured in dB per percent impedance change at the middle two thirds of each timulus-response curve The values plotted in the graph to the right in I g 7 represent the differences between th figures deri ed from the timulus-res onse curves obtained bef re and after the administration of Nembutal It i seen that the crossed reflex (filled circles) i much more ffected than the uncrossed one (open circles)

A slight decrease in excitability of the reflex was found even after doses a low 1.5 mg kg It hould be mentioned that the effect of a certain

dose on the reflex showed individual variations probably due to differences in the absorption of the drug. A subjective rough estimate of the degree of intoxication was quite well correlated to the amount of depression of the reflex.

DISCUSSION

The results of the present study show that both ethanol and pentobarbital sodium have a depressive effect on the crossed and the uncrossed acoustic middle ear reflexes. Although the anatomy of the crossed and uncrossed reflexes is not known in detail, the neuronal organization of the crossed reflex can be considered to be more complex than that of the uncrossed. A functional difference is demonstrated by the fact that the uncrossed reflex can be activated by a lower sound level than the crossed reflex (Møller 1961, 1962 *b*). Furthermore the present study indicates that the uncrossed and crossed reflexes are equally sensitive to ethanol whereas the contralateral reflex is more susceptible to Nembutal than the ipsilateral one.

The effect of ethanol and pentobarbital on spinal reflexes has been studied by several investigators. According to the experiments on decapitated cats (Kolmodin, 1953) there is no significant difference in the sensitivity to ethylalcohol of the polysynaptic and monosynaptic reflexes within the dose range used in the present study. Only at higher doses was a difference seen, the polysynaptic reflex being more influenced than the monosynaptic.

As mentioned above, the crossed middle ear reflex presumably has a more complex central background than the ipsilateral, and our findings on the effect of Nembutal are thus in general agreement with those obtained in experiments on spinal reflexes which show that polysynaptic reflexes are more sensitive to barbiturates than the monosynaptic ones (Wikler & Frank, 1948, Petersén 1952).

The slope of the stimulus-response curve for the crossed middle ear reflex is markedly diminished after Nembutal which indicates that the effect on the reflex is greater well above threshold than near threshold (Fig. 6). The slope of the ipsilateral stimulus response curve is only slightly decreased after Nembutal. Ethanol does not produce such an effect except at doses above a blood alcohol concentration of 0.12% where a slight decrease in the slope is seen both for the crossed and uncrossed reflex. Rall (1955) and Somjen (1963) studied the stimulus-response relation for the monosynaptic spinal reflex before and during barbiturate anesthesia. They found that the slope of the stimulus response curve sometimes increased and sometimes decreased. However Rall and Somjen expressed the stimulus intensity as "effective input" represented by the magnitude of the ventral root synaptic potential whereas in our study the input is represented by the intensity of the natural stimulus.

The difference between the action of ethanol and Nembutal on the crossed

middle ear reflex is of interest with regard to the proposed joint action of these two drugs. Our results favour the view of a different mode of action on the CNS of the two substances and of independent depressor effects, as has been suggested (Aston & Collumbine, 1959)

It is generally accepted that the acoustic middle ear reflex protects the inner ear against sounds of high intensity A reduction of 10 dB in the sensitivity of the reflex as a result of a blood alcohol concentration of about 0 13% brings down the protective action of the middle ear reflex significantly and thus correspondingly lowers the damage risk criteria of sounds.

ACKNOWLEDGMENT

This work was supported by the Swedish Medical Research Council, Stiftelsen Therese och Johan Andersson Minn and by F Ikeans forskningsfond. We are indebted to The Government Laboratory for Forensic Chemistry (Head Professor R. Bonni hsen) for determining th blood alcohol con entration

ZUSAMMENFASSUNG

Die Einwirkung vo Äthylalkohol und Pent barbital-N auf den kustischen Mitt lohrreflex beim Menschen wurd untersucht durch Bestimmung der Än derung der kustischen Impedan des Ohres vor und nach oraler Administration von Äthylalkobol fur Blutalkoholkonzentrationen von 0 02 bis 0 15% und von Pentobarbital-Na in Dosieru gen von 1,6 bis 4,3 mg/kg. De Reflex wurd durch Impulse von reinen Tönen n 500 bzw 1450 Hz usgelöst u d seine Einwirk ng auf beide Ohren gleichze tig kontinuierlich aufgezeichnet und zwar f0 Beschallung einmal des rechten Ohres u d dann des linken Ohres. In dieser Weise k nnt die Auswirkung der Drogen sowohl uf d n Reflex des beschallten Ohres wie den des Gegenohres untersu ht werden

E zeigt ich, dass sowohl Äthan l wi Pentobarbital-N ine hemmend Wirkung auf den Reflex haben. Im einzelnen konnte festgest llt werden, dass der Reflex durch Äthanol auf dem beschallten Ohr und uf dem Gegenohr in gleicher Weise beeinflusst wird während Pentobarbital-Na in stärkerem Masse auf den Reflex des Gegenohres als den des beschallten Ohres einwirkt. Des weiteren zeigt ich, dass mit wachsender Intensität der Beschallung die Stärk des Reflexes auf dem Gegen h bei Pentobarbital u in vermindertem Grade zunimmt, während Äthan l in dieser Hinsicht k ine Einwirkung a f den Reflex sowohl des beschallten Ohres wie des Gegenohres erkennen lässt. Abschliessend werden d Ergebn sse kurz m Zusammenha g mit den Befu den der Einwirkung von Äthanol und Ba b turat uf die spinalen Reflex erörtert und desglei hen auch die F lgerungen der durch die Drogen bewirkten erminderten Mitt lohrmuskelkt t t hinsichtlich Schallschädigungen des Innenohres.

REFERENCES

A s, R nd Cullumbine, H., 1959: Studies on the nat re of the joint action f ethanol nd barbit rates. Toxicology 1 65.

Carr, J H nd Smith, C. M 1962 Effect of some central nervous sy tem depressa t on he phasic nd tonic stretch reflex J Pharm. E p Therap 136 276

HEINBECKER, P and BARTLEY S H 1940: Action of ether and Nembutal on the nervous sy tem. *J Neu ophysiol 3* 219

HOFFMEISTER, F 1962: Über cerebrale polysynaptische Reflexe des Kaninchens und ihre Beeinflussbarkeit durch *Pharmaka Arch Int Pharmacodyn 139* 512.

JEPSEN O 1955: *Studies on the Acoustic Stapedius R flex in Man.* Thesis. Aarhu Univ rsitet, Universitets forlaget i Aarhus, Denmark, 1

KLOCKHOFF I 1961: Middle ear muscle refle es in man. *Act Otolaryng* (Stockh.), Suppl *164*

KOLMODIN G M 1953 The acti n of ethyl alcohol on the monosynaptic extensor reflex and the multisynaptic refl *Acta Physiol Scand 29* Suppl *106* 530.

LAWRENCE, M 1960: Acoustic effects of middle ea sub titution. *Trans. Amer Acad. Ophthal Ot laryng 64* 23.

METZ, O 1951 Studies on the contraction of the tympanic muscles as indicated by cha ges in the impedance of th ear *Acta Otolaryng* (Stockh) *39* 397

MITCHELL, C. L., 1964 A comparison of drug ffect upon th J w jerk response to electrical stimulation of the tooth pulp in dogs nd cats. *J Pharm Exp Therap, 146* 1

MØLLER, A 1958 Intra aural muscle contracti n in man, examined by measuring acoustic impedance of the ear *Laryngoscope 68* 48

— 1961 Bilat ral contracti n f the tympanic muscles in man examined by measuring acoustic impedance-change *Ann Otol 70* 735

— 1962 *a* Th sensitivity of contraction of the tympani muscles in ma *Ann Otol 1* 86

— 1962 *b*: Acoustic refl x in man. *J Acoust Soc Am 34* 1524

— *1964: Effect of tympanic muscle acti ity on m vement of the eardrum,* acoustic impedance and cochl r microphonics. *Acta Otolaryng* (Stockh) *58*, 525

PETERSÉN I 1952 Differences in sensiti ity to naesthetics of mot r centres in the cervical and lumba regi n f the spinal cord. *Acta Physi l Scand 26* Suppl. 96

HALL, W 19 5: Expe imental m nosyn ptic inp t-output rel ti ns in the mammalian pinal cord *J Cell C mp Phy i l 46* 413

SIOG, E. B HOLLAND, R nd SCHNEIDER, J A 1958 The influ n of om n barbiturate depressa ts on central polysynapti mechanisms *Arch Int Pharmacodyn 116* 434.

SOMJEN G G 1963: Effect of th nd thi p ntal on pinal presynaptic terminals. *J Pharm Exp The ap 140* 396

WE SALL, R 1958 The tympanic m scles d thei refl xes. *A ta Ot laryng* (Stockh.) Suppl *139*

WIKLER, A., 1945: Effect f morphin N mbutal, ther and ese ine n two-ne ron and multi uron flex in the cat *Proc Soc Exp Biol* (N Y) *58* 193

WI LER, A nd FRANK, K 1948: Hindlimb refl xes of chronic pi al dogs during cycles f addi ti n t morphi and methadon *J Pha m Exp Th rap 94* 382.

WITKIN L B S ITALETTA, P nd PLUMMER, A J 1960 Th ffect f some ce tral depressa t on spinal refl xes of the i tact n th ed cat *Arch Int Pharmacodyn 124* 105

Dept f Phy i logy K linska In titutet
Stockh lm 60 Swed n

Received March 14 1967

Acta oto-laryngologica 64 427 434 (1967)

VASCULAR STUDIES IN MENIÈRE'S SYNDROME AND DISEASE

F L. JENKNER and E. VOJACEK
Graz Austria

From the Departments of Surgery (Head Prof F Spath) and Otolaryngology (Head Prof W Messerklinger) the University of Graz

The hypothesis of neurovascular dysfunction of the blood supply t the labyrinth was tested clinically Excluding other pproaches, an evalua tion f erebral hemodyn mics by rheoencephalography was made on 200 patients suffering from Menière s disease or showing evidence of Menière syndrome or similar ymptoms. The results are compared with those of other pertinent work such a cardiovascular neurologic and otologic examinations, CSF-study EEG and x-rays of the skull, pyramid and cervical spine Four tables summarize the essentials of these findings. Rheoencephal graphy allowed differentiation between groups of patients with cerebral arteriosclerosis, cerebrovascular in-suffi iency a l (unilateral) cerebrovascular damage This la t group coincided entirely with the diagnosi of genuine Menière s disease These result confirm the aforementioned hypothesis. They also establish rheoencephalography as a desirable and critical method for diagnosing Menière's disease At the same time a new appro h for treating Menière's disease in it acute stage i suggested

INTRODUCTION

Vasomotor di turbances of a neurasthenic type are affecting the vessels upplying the inner ear and causing Menière s disease" Oppenheimer (1923) made this apodictic statement at the beginning of the century Less frequently he credited atheromatosis f these vessels as a cause of Menière s disease ba ing his opinion n an autopsy His belief about neurasthenic asomot r disturbances was f unded on the frequent observation of blushing of the face and arm during an cute attack of Menière s disease Many uthors ha had similar thoughts (Fowler & Zeckel, 1952–53 Hilger 1950 Oppenheimer 1923 Altm nn, 1955)

T d y w are living in n era of bjectivation. Surmises nd opinions r t regarded highly unles based on t tistically significant" observa tion Thi does not diminish the beliefs of experienced clinicians. The value of precise observation leading t uch belief is frequently confirmed by the objecti e scientific method nd approach of our time This may truely be our tribute to the ing nious observing mind of the old clinicians.

Consciou of thi trend toward objecti ity we re fully aware that exact ev lu tion of ascular nd neurogenic factors of the inner ear i impossible

HEINBECKER, P and BARTLEY S H 1940: Action of ether and Nembutal on the nervous system *J Neurophysiol.*, *3* 219

HOFFMEISTER, F 1962 Über cerebrale polysynaptische Reflexe des Kaninchens und ihre Beeinflussbarkeit durch Pharmaka. *Arch Int Pharmacodyn.*, *139* 512.

JEPSEN, O 1955 *Studies on the Acoustic Stapedius Reflex in Man* Thesis. Aarhus Universitet, Universitetsforlaget i Aarhus, Denmark, 1

KLOCKHOFF I 1961 Middle ear muscle reflexes in man. *Acta Otolaryng* (Stockh.), Suppl *164*

KOLMODIN G M 1953 The action of ethyl alcohol on the mon synaptic extensor reflex and the multi ynaptic reflex *Acta Physiol Scand.*, *29* Suppl. *106*, 530.

LAWRENCE, M 1960 Acoustic ffects of middle ear substitution. *Trans. Amer Acad. Ophthal Otolaryng* *64* 235

METZ, O 1951 Studie on the contraction of the tympanic muscles a indicated by changes in the impedance of the ear *Acta Otolaryng* (Stockh) *39* 397

MITCHELL, C. L., 1964 A comparison of drug effects upon the jaw jerk response to electrical stimul tion f the tooth pulp in dog and cats *J Pharm Exp. Therap.*, *146* 1

MØLLER, A 1958 Intra aural muscl contraction in man, xami ed by measuring acoustic imped nce of the ear *Laryng scope* *68* 48

— 1961: Bilateral contraction of the tympanic musel in man xamined by measuring acou tic impedance-chang *Ann Otol* *70* 735

— 1962 *a*: The sensitivity f contracti n of the tympanic muscles in man. *Ann. Otol* *71* *86*

— 1962 *b* Acoustic reflex i ma *J A oust Soc Am r* *34* 1524

— 1964 Effect of tympanic muscle acti ity on movement of the eardrum, coustic impedance and cochlear microph nics. *Acta Ot laryng* (Stockh.) *58* 525

PETERSÉN I 1952: Differences in sensitivity t an esthetics of motor ce tres in the cervical nd lumba region of the pinal cord *A ta Physiol Scand* *96* Suppl *96*.

R LL, W 1955: Experimental mono ynapti input–output relations in the mammalian spin l cord. *J Cell Comp Phy i l* *46* 413

SIGG, E. B HOLLAND R nd SCHNEIDER, J A 1958: The infl ence of som n barbiturat depressant n central polysynapti mechanisms. *Arch Int Pharmacodyn* *116*, 450.

SOMJEN, G G 1963: Effects f ether nd thiopental on spinal presynaptic terminals. *J Pharm Exp Therap* *140* 396

WERSÄLL, R 1958 The t mpani muscl nd thei reflexes. *Acta Otolaryng* (Stockh.) S ppl *139*

WIKLER, A 1945 Effects f morphine Nembut l, ether nd eseri on two-ne ron and multi ron refl x in the cat *Proc Soc Exp B l* (N Y) *58* 193

WIKLER, A nd FRANK, K 1948 Hindlimb refl es of hroni pinal dogs du ing cycles f ddicti n t morphin d m thad n *J Pharm Exp Therap* *94* 382.

WITKIN, L. B SPIT LETTA, P a d PLUMMER, A J 1960 Th effect f some central depressa t on pinal refl xes f the i tact th ized t *Arch I t Pharmacodyn* *124* 105

D pt of Physi logy Karolinska Institutet
Stockholm 60 Swed n

Received Ma ch 14 1967

TABLE 1

Symptom	Incidence among all patients (cases)	Incidence among patients with Menière disease	
		Yes	No
Loss of hearing	93	33	16
Vertigo	85	38	11
Tinnitus	61	23	26
Headache	53	14	35
Vomiting	41	20	29
Nausea	31	12	37
Disturbed equilibrium	15	7	42

Rheoencephalographic reports were typed without any knowledge of the clinical history symptomatology or results of other clinical or laboratory tests. Each report was given a number corresponding to a patient. After entering the semiquantitative evaluation of REG on a roster (by number not name) a third party entered the respective results of all other (numbered) examinations in the same roster Since this person had no knowledge of the meaning of the various tests, an unbiased correlation of results was obtained

RESULTS

It was decided not to present the entire roster of 200 patients, which would be cumbersome to survey but to make a tabular summation of the results of the majority of the examinati ns. Thus, even though some details m y be lost, the more important results are readily evident and certain conclusions easier to comprehend.

Table 1 gives the symptomatology divided into incidence among all patient and incidence or none-existence of symptoms in the group with Menière s disease Table 2 lists the results of various examinations, grouped s to radiological, blood cardiovascular ophthalmological and neurological ex minations. All pathological findings are summed up into one column (e.g. high a well a low blood pres ure etc) If the finding was not noted, not noted clearly enough or if the test was not done this is entered in the third column From thi tabular composition, it seems to us that one of th examination which is of greatest value in evaluating patients with Menière disease should be x-ray of the cervical vertebral column (41 pathological ersu 13 or 19 in other radiological examinations) Ophthalmological ex mination i nece sary t distinguish several rare disease entities with imil r symptomat logy In our 200 patients, there were two with prov n brain tumor one bronchogenic carcin ma with cerebral met tases, and two cases f trigeminal neuralgia diagnosed by our screening technique

at this time due to lack of precise anatomical and physiological knowledge Therefore, we submitted 200 patients (some of them with Menière's disease the majority with one of the pertinent symptoms) to an examination of cerebral hemodynamics. We are of the opinion that the internal auditory artery and cerebral arteries should be influenced similarly if not in the same way by any neurasthenic vasomotor disorder A preliminary study required this clinical investigation on 200 patients, the results of which are reported here

MATERIAL AND METHOD

Among the selected 200 patients of the Department of Otolaryngology of the University of Graz were 49 afflicted with genuine Menière's disease the other patients included were chosen according to similar symptoms, such as the sudden onset of loss of hearing or increasing loss of hearing (inner ear) with some cases of tinnitus and vertigo The purpose of this choice was to exclude any possible bias in the person evaluating the vascular examination These patients were subjected to a group of tests. Some patients underwent all tests, whilst others had only some of those mentioned below In addition to noting age sex, height and weight, blood counts, blood pressure, Schellong test, blood sedimentation rate urinalysis, serological (Wassermann) tests and, if deemed necessary cardio-pulmonary evaluation were carried out Ophthalmological status included ophthalmoscopy—sometimes visual fields and retinal artery pressure Neurological examination included cerebrospinal fluid sampling electroencephalography and occasionally air studies or angiograms of the carotid or vertebral arteries. Otological studies included audiograms and vestibular testing, when necessary

Examination of cerebral hemodynamics was done on all patients by the method of "rheoencephalography' (REG) This is an indirect electrical method of evaluating cerebral circulation by observing and graphically recording the changes in electrical resistance to an alternating current by means of Wheatstone bridges, one for each hemisphere To exclude interferences between the two fields, the apparatus used (and commercially available Doppelrheograph, Dr Schuhfried) maintains a stable frequency with a set difference of frequencies between the two fields. In brief changes in blood content (volume pulse) of any part of the body influence electrical resistance to an alternating current (of an approximate frequency of around 30 000 cps in this case) which changes are then rectified amplified and recorded graphically and continuously Details of the method are described elsewhere (Jenkner 1962) Square aluminum block electrodes are placed over saline soaked pads on the forehead both right and left, parasagittally and over both mastoids. One frontal and one mastoid electrode are used to establish one field The changes in resistance thus observed correspond mainly (for practical purposes only) to changes of cerebral hemodynamics (Jenkner 1962)

TABLE 3

Patients	Methods	Normal	Right	Left	Bilat.	Other
All	Otological	14	37	40	48	—
All	REG	25	60	77	36	12
All	EEG (focal slowing)	78	9	16	—	29
Menière	Otological	—	26	23	—	—
Menière	REG	—	27	22	5	—
Menière	EEG	27	2	3	—	5
Arterioscl.	EEG	17	3	8	—	4

TABLE 4

Criteria	Diagnosis	Patient
Otological	Menière	49
REG	Normal	25
	Borderline	12
	Vascular insufficiency	35
	Vascular damage	64
	Arterioscl. (unilat 19, bil t. 21)	40
	Not classified	34
EEG	Normal	78
	Slight diffuse abnormal	22
	Focal slow waves	25
	Suspected epilepsy	6
	Atypical migrain	1

Table 4 summarizes the various diagnostic groupings under the three most important criteria Otological-clinical (without any clear-cut diagnosis except Menière) the reason for this being that the other cases were pur posely submitted to EEG-examination because of some similar features, but having other pathology of varying nature Rheoencephalographic diagnosi was normal and borderline 37 times vascular insufficiency 35 times vascular damage (the same patients a those under the otological clas ification of "Menière" and 5 bilaterals) 64 cases and arteriosclerosis, 40 times. N t classified because of artefacts in the records (new technician) multiple extra sy les, and other reason were 34 cases included in this grouping are two cases which uggested cerebral compression, which were further evalu ted by carotid arteriography and diagnosed finally a cerebral tum rs. Electroencephalographic findings are separated into "Menière" and arteriosclerosis" We hall n w turn to the significance and meaning these findings may ha e

TABLE 2

Examination	Results		
	Normal	Pathological	Not noted
Roentgen examination of			
Skull	98	13	89
Cervical spine	39	41	120
Pyramids	68	19	113
Blood count, compl	68	46	86
Blood pressure	79	58	63
Schellong	14	42	144
Ophthalmoscopy	130	16	54
Visual fields	11	1	188
Retinal artery pressure	16	1	183
Neurological	69	66	65
Cerebrospinal fluid	53	8	159
Electroencephalogram	78	54	52
Rheoencephalogram	25	163	(Borderline 12)
Audiometry	8	138	54
Vestibular function	31	60	109

To assess the relative value of neurological and rheoencephalographic techniques an evaluation of the possibility of lateralizing correctly without reference to otological findings compared with otological-clinical lateralization is necessary—Table 3 From this table it becomes evident that comparing the sides of lesions in all our cases is irrational due to the great difference in numbers of patients subjected to the various examinations exact otological-clinical classifications were possible in 130 cases. Rheoencephalographic classification was possible in 188 cases, not including the 12 borderline cases Electroencephalographic evaluation was obtained in 122 cases. The relatively similar total number of patients in otological and EEG-classification would suggest a possible comparison, but here there are great differences in the results already the two numbers for normals are 14 and 78 respectively This may be due to the fact that not all patients had both of these examinations

Comparing the numbers for the genuine Menière group (otological 49 REG the same 49 plus 5 bilaterals) and adding the EEG-classification (of these patients, 41 had EEG s) it becomes evident that there are no normal REG s among this group of clear-cut pathological cases but there are 27 normal EEG s Focal slowing was reported five times, twice on the right and three times on the left side, while other pathology included three cases with slight abnormality (generalized) and two cases of suspected epilepsy The significance and a possible explanation for these findings will be indicated in the discussion

Table 3

Patients	Methods	Normal	Right	Left	Bilat.	Other
All	Otological	14	37	40	48	—
All	REG	25	50	77	38	12
All	EEG (focal slowing)	78	9	16	—	29
Menière	Otological	—	26	23	—	—
Menière	REG	—	27	22	5	—
Menière	EEG	27	2	3	—	5
Arteriosel.	EEG	17	2	6	—	4

Table 4

Criteria	Diagnosis	Patients
Otological	Menière	49
REG	Normal	25
	Borderline	12
	Vascular insufficiency	35
	Vascular damage	54
	Arterioscl. (unilat. 19 bilat. 21)	40
	Not classified	34
EEG	Normal	78
	Slight diffuse abnormal	22
	Focal slow waves	25
	Suspected epilepsy	6
	Atypical migraine	1

Table 4 summarizes the various diagnostic groupings under the three most important criteria Otological-clinical (without any clear-cut diagnosis except Menière) the reason for this being that the other cases were purposely submitted t EEG-examination because of some similar features, but having other path logy of varying nature Rheoencephalographic diagnosis was normal and borderline 37 times vascular insufficiency 35 times vascular damage (the same patients as those under the otological classification of "Menière and 5 bilaterals) 54 cases and arteriosclerosis, 40 times. Not classified because of artefact in the records (new technician) multiple extrasystoles, and other reasons were 34 cases Included in this grouping are two cases which suggested cerebral compression, which were further evaluated by carotid arteriography and diagnosed finally as cerebral tumors. Electroencephalographic findings are separated into "Menière and "arteriosclerosis" We hall now turn to the significance and meanings these findings may have.

TABLE 2

Examination	Results		
	Normal	Pathological	Not noted
Roentgen examination of			
Skull	98	13	89
Cervical spine	39	41	120
Pyramids	68	19	113
Blood count compl	68	46	86
Blood pressure	79	58	63
Schellong	14	42	144
Ophthalmoscopy	130	16	54
Visual fields	11	1	188
Retinal artery pressure	16	1	183
Neurological	69	66	65
Cerebrospinal fluid	53	8	159
Electroencephalogram	78	54	52
Rheoencephalogram	25	163	(Borderline 12)
Audiometry	8	138	54
Vestibular function	31	60	109

To assess the relative value of neurological and rheoencephalographic techniques an evaluation of the possibility of lateralizing correctly without reference to otological findings compared with otological-clinical lateralization is necessary—Table 3 From this table it becomes evident that comparing the sides of lesions in all our cases is irrational due to the great difference in numbers of patients subjected to the various examinations exact otological-clinical classifications were possible in 139 cases. Rheoencephalographic classification was possible in 188 cases, not including the 12 borderline cases Electroencephalographic evaluation was obtained in 122 cases. The relatively similar total number of patients in otological and EEG-classification would suggest a possible comparison but here there are great differences in the results already the two numbers for normals are 14 and 78 respectively This may be due to the fact that not all patients had both of these examinations

Comparing the numbers for the genuine Menière group (otological 49 REG the same 49 plus 5 bilaterals) and adding the EEG-classification (of these patients, 41 had EEG s) it becomes evident that there are no normal REG s among this group of clear-cut pathological cases but there are 27 normal EEG s Focal slowing was reported five times, twice on the right and three times on the left side while other pathology included three cases with slight abnormality (generalized) and two cases of suspected epilepsy The significance and a possible explanation for these findings will be indicated in the discussion.

TABLE 3

Patients	Methods	Normal	Right	Left	Bilat.	Other
All	Otological	14	37	40	48	—
All	REG	25	60	77	36	12
All	EEG (focal slow) 20	78	0	16	—	29
Menière	Otological	—	26	23	—	—
Menière	REG	—	27	22	5	—
Menière	EEG	27	2	3	—	5
Arterioscl.	EEG	17	3	8	—	4

TABLE 4

Criteria	Diagnosis	Patients
Otological	Menière	49
REG	Normal	25
	Borderline	12
	Vascular insufficiency	35
	Vascular damage	54
	Arteriosel. (unilat. 19 bilat. 21)	40
	Not classified	34
EEG	Normal	78
	Slight diffuse abnormal	22
	Focal slow waves	25
	Suspected epilepsy	6
	Atypical migraine	1

Table 4 summarizes the various diagnostic groupings under the three most important criteria. Otological-clinical (without any clear-cut diagnosis except Menière) the reason for this being that the other cases were purposely submitted to EEG-examination because of some similar features, but having other pathology of varying nature. Rheoencephalographic diagnosis was normal and borderline 37 times, vascular insufficiency 35 times, vascular damage (the same patients as those under the otological classification of "Menière" and 5 bilaterals) 54 cases and arteriosclerosis, 40 times. Not classified because of artefacts in the records (new technician), multiple extrasystoles, and other reasons were 34 cases. Included in this grouping are two cases which suggested cerebral compression, which were further evaluated by carotid arteriography and diagnosed finally as cerebral tumors. Electroencephalographic findings are separated into "Menière" and "arteriosclerosis". We shall now turn to the significance and meanings these findings may have.

DISCUSSION

When discussing these results we want to keep in mind the reason for planning this study. We wished to put to a clinical test the hypothesis of neurovascular dysfunction of the blood supply to the labyrinth. This approach already excluded the electroencephalogram as a potent and reliable diagnostic criterium since the electrical activity of the brain is no criterium for small changes in cerebral hemodynamics. Certainly it is true that marked disturbances of cerebral circulation may influence cerebral metabolism and consequently lead to pathological electroencephalograms. This applies mainly to arteriosclerosis. In our series, among the 40 arteriosclerotic records, 32 EEG's were recorded; of these 17 were normal, 11 showed focal slow waves, 2 slight generalized abnormality and in one case the record was interpreted as suspected epilepsy. In one record atypical migraine was the diagnosis. This is a greater number of pathological records than was reported for the cases of Menière's disease (10 pathological records with only five slow wave foci—see under Results and Table 3).

According to Hilger (1950) vasomotor labyrinthine ischemia is thought to represent the causative mechanism of Menière's disease. This neurovascular dysfunction may also be caused by psychosomatic factors (Fowler & Zeckel 1952–53). In this manner slowing of blood flow is thought to occur just as in the so-called spastic atonic vascular complex of Mueller (1930). Blood sludging (Knisley *et al.*, 1947) is usually seen in these instances. These lines of thought have caused changes in the approach to surgical therapy (Castellano, 1951) of Menière's disease and attempts at studying the effect of sympathectomy on the inner ear (Lewis, 1951). It must be said that the anatomical and pathophysiological assumptions behind these vascular theories remain too difficult a problem to-day to allow a direct study (Altmann 1955) mainly due to lack of knowledge in the respective fields. But the relief of blood sludge by intravenous application of low molecular dextran has been studied (Jenkner in print) elsewhere in the body by the rheographic method and has been observed directly in animal experiments. A study is therefore suggested to discover if the application of this method to the hemodynamics of the brain (called rheoencephalography (Jenkner 1962) might not allow an observation of the continuous changes in cerebral circulation. Parallelism between cerebral and inner ear hemodynamics is to be suspected when similar findings occur in the conjunctiva of some psychosomatic affections, the more so since at least a partial psychosomatic background is postulated by some workers for the disease in question (Fowler & Zeckel 1952, Oppenheimer 1923).

In using our indirect method (REG) of evaluation of cerebrovascular changes, three groups of diagnostic terms (Table 4) are rather closely correlated. These are cerebrovascular insufficiency, vascular damage and cerebral arteriosclerosis. The latter group is the most clearly distinguishable

as has been frequently demonstrated (Jenkner 1962) Cerebrovascular insufficiency was diagnosed by the tracings in only 24 cases. In the remaining 11 cases data on blood pressure and/or blood count had to be considered. In these cases, the record could be classified accordingly because of either low systemic blood pressure and/or severe anemias. Had it not been for these additional pieces of information, the distinction between this record and the group "vascular damage" would have been rather difficult In the group of vascular damage the damage was found to be unilateral in 49 cases. These cases turned out to be exactly the cases with the clinical otological diagnosis of "Menière The five others in this category presented vascular damage bilaterally and could not be given a clinical diagnosis.

It is interesting to note that the group of cerebrovascular insufficiency had similar symptoms to the group of vascular damage One may think of these patients as having had the arterial hypotension for a long time of their anemia having developed gradually or of it having been present always therefore the clinical episodes of dizziness and now and then, fainting spells that invariably must have been present should be easily *recognizable clinically* Sometimes, these patients presented headaches and *tinnitus. In* view of these symptoms, they were included in the study after *the first* examinations were carried out, the initial clinical impression was confirmed and it seems natural for an examination of the cerebrovascular state to recognize these cases immediately especially when data on BI and RBC are available for reference In this group of patients, local differences exist in cerebral circulation in addition to generalized changes, which prevail unilaterally in the group of vascular damage, to which we will now turn.

This study was undertaken to observe the cerebral hemodynamics in these patients. The titl was chosen only with hesitation We agree entirely with the opinion of Altmann (1955) that the term "Menière's syndrome" *is* often used in a misleading way However in view of the subtle problem of those vascular factors that several theories seem to assume to be responsible for labyrinthine hydrops, it was necessary to examine not *only* patients with "Menière's disease In addition, patients with similar symptomatology but other diseases, had to be included in a double blind manner to obtain pertinent and objective data

For registering hanges of such nature in cerebral hemodynamics, one need not be concerned with small hanges in on or the other specific areas. If thi parallelism exists, *all of the* vertebrobasilar supply areas ought to be inv lved E en the area supplied by the carotids might be affected in the sam way but not necessarily To observe changes in one or the other cerebral hemispheres, rheoencephalography in its standard derivation with electrod placements *frontally* and retromastoideally on either side is definitely the optimal method availabl to-day There is only one prerequisite for this electrical (indirect) method of observing cerebrovascular changes the apparatus used must conform with certain standards mentioned else

where (Jenkner 1962) We know of several designs of available apparatus which meet these standards but we also know of some that do not. We are also aware of apparatus not representing bridge circuits which is reliable and other equipment that is entirely unreliable and not suited for our purpose (for reasons which do not interest us here) of observing cerebral hemodynamics and its changes Rheoencephalography as elaborated by one of us (F L J) and confirmed by many other workers, has only one apparent disadvantage for our study—due to the field distribution (which was measured in animals and models as well as in some patients) records which represent the changes of the part of the brain supplied by the carotid circulation to a much greater extent than the vertebral basilar supply area (Jenkner) This, at first seemed to be a disadvantage However since one may not expect to obtain an electrical counterpart of the internal auditory artery supply by rheoencephalography but rather the overall cerebral hemodynamics and changes in its parallelism to the hemodynamics of the inner ear the results evidently confirm our supposition After the preliminary study a failure rate of 10% was expected which was not even reached The results show a disagreement in only 4% of the group of genuine Menière s disease

Our results seem to confirm the opinion of Hilger (1950) and others (Altmann, 1955 Fowler & Zeckel 1952–53) We are aware of the indirect nature of the evidence we obtained It is true of all research work, how ever experimental or clinical that results stimulate investigations of a more direct, more exact nature. These usually surpass previous studies. With this investigation we hope we have contributed to the controversial question of vascular causes for Menière s disease Until we are able to in vestigate this subject by better means it is our belief that studies of this nature on a larger series of patients may point the way to a rational therapy of this disorder

Thought alo g these lin m y h e prompted Losel (Lösel, H Behandlung n Durchblutu gsstörung n mitt l D uertropfinf ion n Rheomakrod x® 10.) t include i hi report on 52 cases 3 pati nts with Menière syndrome He does not gi i dications to the e t examin ti n f hi three cases r does h m nti n why he included cases with thi di gnosis. He only report th res lt i all of these three cases a cell t Only afte thi cli i al heoencephal graphi tudy w s concl ded did we start to cons der therapy with multipl i fu i ns f low m lecula w ight d xtran nd are n w follow g these case l sely

ZUSAMMENFASSUNG

Die Hypothese der neuro-vaskulären Dysfunktion der labyrinthären Blutver sorgung wurde einer klinischen Prüfung unterzogen Mangels anderer Methoden wurde die cerebrale Haemodynamik mittels der Rheoencephalographie in 200 Patienten mit Menière s Krankheit und Syndrom untersucht Die Ergebnisse wurden jenen aller einschlägigen Untersuchungen wie cardiovaskuläre neurolo gische und otologische röntgenologische (Schädel Pyramiden und HWS) Liquor

und EEG-Untersuchungen, gegenübergestellt. Die wichtigsten Befunde wurden in 4 Tabellen zusammengestellt. Es zeigte ich daß das Rheoencephalogramm zwischen Patienten mit Arteriosclerose, cerebro-vaskulärer Insuffizienz und Hirngefäßschad (unilateral) unterscheiden kann. Die letzte Gruppe wurde als identisch mit jener der genuinen Menièreschen Krankheit gefunden. Die Ergebnisse bestätigen die gena te Hypothese und machen die Rheoencephalographie zu einer erstrebenswerten Untersuchung in d Diagnose der genannten Er krankung. Gleichzeitig sch int ein neuer Weg in der Therapi dieser Erkrankung aufgezeigt.

REFERENCES

Altmann, F 1965 Morbus Menière *Fortsch Hal Nas. Ohrenheilk* 2, 1 S. Karger Basel/New York.

Cawthorne(?), F 1931 Menière disease nd its surgical treatment. Report f 300 cases. *J N eurosurg* 8, 173.

Fowler, E. P Jr., nd Zeckel, A 1952 Psychosom tic pects of Menière disease *J Am Med Ass.*, *148* 1265.

— 1953 Psychophysiological factors in Menière' dise se *Psychosom Med* 15 127

Hilger, J A., 1950 V somotor labirinthine ischemia *Ann. Otol* 59 1102.

Jenkner, F L., 1962 Zur Durchblutungsstörung on H rn nd I ne ohr *Mschr Ohrenheilk.*, 96 472.

— 1962 *Rheoencephalography A Method f the Continuous Registration f Cerebrovascular Changes.* C. C. Thomas, Spri gfield, Ill., U.S.A (America Lecture Series I Neurology No. 472.) Translator Russia ed., Moscow 1966

— 1965 Orientierende U tersuchung uber die haemodynamischen Verhält hse durchblut gegestörten Extremitäten peripher en G f sverschlüssen. *Z Kreislaufforschung* 54 300

— Unpublished data

Kinsley, M. E., Eliot T S., Bloom, E., nd Wan er, L., 1947 Sl dged blood. *Scienc* *106* 431

Lewis, R. S 1951 Sympathectomy nd the I ner ea *J Laryng* 65 825

M ller, O 1939 *Die fein I Blutgefässe des M nsche in gesund n und kranken Tage* 2nd ed. Enke Stuttgart.

Oppenheimer, H 1923 *Leh buch der N rvenkrankh i* Part II pp. 1880–1882. S. Karger Berl n.

Doz. Dr F L. J nkn
Fichtnergasse 22,
1130 Wi XIII
Austria

Rece ved March 22 1967

Acta oto-laryngologica 64 436–448 (1967)

ANATOMICAL DISTRIBUTION OF EFFERENT FIBERS IN THE VIIITH CRANIAL NERVE OF THE BULLFROG (*RANA CATESBEIANA*)

RUTH G ROBBINS, M S., R S BAUKNIGHT B S., and V HONRUBIA, M D.
Nashville Tenn., U.S.A

From the Division of Otolaryngology Division of Hearing and Speech Pathology and the Department of Physiology The Bill Wilkerson Hearing and Speech Center and Vanderbilt University School of Medicine Nashville

The VIIIth cranial nerve of the bullfrog was surgically transected between the brain stem and sensory ganglion Two to three weeks after section of the nerve the frogs were sacrificed and the Wallerian degeneration in the nerve was studied It was possible to demonstrate the presence of efferent fibers going toward the sensory epithelium of all the recognized vestibular end-organs, and also toward the papilla of the pars amphibiorum However no efferent fibers were seen at the level of the papilla basilaris. The ganglion cells of both the acoustic and vestibular afferent fibers appeared normal Many degenerated fibers were seen around the sacculus wall among a group of fibers previously undescribed

The structure of the frog s ear has been studied over a long period of time The gross anatomy of the sensory areas of the inner ear and their nerve supply from the peripheral divisions of the VIIIth cranial nerve have been identified but the detailed anatomical structure and the physiological significance of the different sensory receptors is not clearly established A recent review by Geisler van Bergeijk & Frishkopf (1964) makes reference to the earlier work of Kuhn (1880) and Retzius (1881) whose drawings have been most helpful in the present study

Rasmussen (1946) demonstrated the existence of an efferent innervation in the cochlea by classical anatomical methods. Subsequent works using a variety of techniques have defined the course and distribution of these fibers in the inner ear (Fernández, 1951 Portmann *et al* 1953 Rasmussen, 1953 Petroff 1955 Smith & Dempsey 1957 Dohlman *et al* 1958 Engström, 1958 Engstrom & Wersall 1958 Rasmussen & Gacek, 1958 Schuknecht *et al.*, 1959 Gacek, 1960 Rasmussen 1960 Boord, 1961 Gacek, 1961 Ireland & Farkashidy 1961 Rossi 1961 Smith & Sjöstrand, 1961 Hilding & Wersall 1962 Iurato, 1962 Kimura & Wersäll 1962 Rossi & Cortesina, 1962 Smith & Rasmussen, 1963 Spoendlin & Gacek 1963 Gacek *et al.*, 1964 Rossi & Cortesina 1965 Smith & Rasmussen,

This work was supported in part by the United States Public Health Service Grant NB 05690-02, NB 05674-03 and The Deafness Research Foundation

1965) The function of the efferent system of fibers in the cochlea and vestibular sensory end-organs has been postulated by different authors (Galambos, 1956 Desmedt, 1960 Fex 1962 Desmedt & La Grutta, 1963 Gleisner & Henriksson, 1963 Schmidt, 1963)

In the frog the presence in the VIIIth nerve of some fibers of centrifugal nature has been demonstrated by electrophysiological methods (Schmidt 1963 Gleisner & Henriksson 1963) From terminal twigs dissected from the small branches of the vestibular receptors Schmidt (1963) was able to record efferent impulses going toward the three ampullae the sacculus, utriculus and lagena of the Rana pipiens. This work was the first electrophysiological evidence of the existence of efferent innervation to the vestibular apparatus in any animal However the anatomical evidence of the existence of these fibers in the frog is still lacking.

This study was designed to investigate the anatomical distribution of the efferent system of nerve fibers in the inner ear of the frog The method adopted, similar to the one used by Petroff (1955) Rasmussen & Gacek (1958) and Gacek (1960) consists of observing the presence and extent of Wallerian degeneration in the peripheral segment of the VIIIth nerve after surgical transection of the root of the nerve between the brain stem and the sensory ganglion.

METHOD

Healthy adult bullfrogs were anesthetized by immersion in 0 10% solution of Tricaine Methylsuphonate. The anesthetized state was maintained during surgery by covering the frog with gauze moistened with the solution The frog was placed on its back on the dissecting board with the nose secured t the board and the lower jaw retracted with a clip Surgical procedures were performed with the aid of a dissecting microscope

A midline incision was made in the mucous membrane of the roof of the mouth to expose the area of the parasphenoid bone The muscle overlying the right lateral wing of this bone was pushed back sufficiently to provide a clear field for drilling the bone over the area f the acoustic foramina and exposing the VIIth and VIIIth cranial nerves as they emerge from the brain stem. Gentle suction was used to maintain a clear operating field. Sufficient bone was removed to expose the anastamosis between the VIIth and VIIIth nerves, but not so far as to risk damage to the saccular wall. Care was taken to avoid damage to a tortuous artery passing along the surface of the VIIth nerve nd running across the anastamosis toward the VIIIth nerve Transection of the VIIIth nerve was accomplished by firmly pinchi g the trunk with watchmakers forceps medial to the anastamosis and a lose t the brain stem as possible The area of removed bone wa replaced by a piece of gelfoam. The mucous membrane of the palat wa closed with utures. Transection f the nerve was carried out in all frog on th right ide with the left side serving as a control

Acta oto-laryngologica 64 436–448 (1967)

ANATOMICAL DISTRIBUTION OF EFFERENT FIBERS IN THE VIIITH CRANIAL NERVE OF THE BULLFROG (*RANA CATESBEIANA*)

RUTH G ROBBINS, M S., R S BAUKNIGHT B S., and V HONRUBIA, M D
Nashville Tenn., U.S.A

From the Division of Otolaryngology Division of Hearing and Speech Pathology and the Department of Physiology The Bill Wilkerson Hearing and Speech Center and Vanderbilt University School of Medicine Nashville

The VIIIth cranial nerve of the bullfrog was surgically transected between the brain stem and sensory ganglion Two to three weeks after section of the nerve the frogs were sacrificed and the Wallerian degeneration in the nerve was studied It was possible to demonstrate the presence of efferent fibers going toward the sensory epithelium of all the recognized vestibular end-organs, and also toward the papilla of the pars amphibiorum However no efferent fibers were seen at the level of the papilla basilaris The ganglion cells of both the acoustic and vestibular afferent fibers appeared normal Many degenerated fibers were seen around the sacculus wall among a group of fibers previously undescribed

The structure of the frog s ear has been studied over a long period of time The gross anatomy of the sensory areas of the inner ear and their nerve supply from the peripheral divisions of the VIIIth cranial nerve have been identified but the detailed anatomical structure and the physiological significance of the different sensory receptors is not clearly established. A recent review by Geisler van Bergeijk & Frishkopf (1964) makes reference to the earlier work of Kuhn (1880) and Retzius (1881) whose drawings have been most helpful in the present study

Rasmussen (1946) demonstrated the existence of an efferent innervation in the cochlea by classical anatomical methods. Subsequent works using a variety of techniques have defined the course and distribution of these fibers in the inner ear (Fernández, 1951 Portmann *et al.*, 1953 Rasmussen, 1953 Petroff 1955 Smith & Dempsey 1957 Dohlman *et al* 1958 Engstrom 1958 Engstrom & Wersäll, 1958 Rasmussen & Gacek, 1958 Schuknecht *et al.*, 1959 Gacek, 1960 Rasmussen, 1960 Boord 1961 Gacek, 1961 Ireland & Farkashidy 1961 Rossi 1961 Smith & Sjöstrand, 1961 Hilding & Wersäll 1962 Iurato, 1962 Kimura & Wersäll 1962 Rossi & Cortesina, 1962 Smith & Rasmussen 1963 Spoendlin & Gacek, 1963 Gacek *et al* 1964 Rossi & Cortesina, 1963 Smith & Rasmussen

This work was supported in part by the United State Public Health Service Grant NB 05690–02, NB 03474–03 and The Deafness Research Foundation



Fig. 1. Cross section of the brain stem, VIIIth nerve and inner ear of the bullfrog. The area where the nerve was sectioned is demonstrated by the arrow. Scar tissue bridges the gap. The ganglion cells (*G*) and their nerve fibers are intact. Pars basilaris (*B*). Lagena (*L*).

of demonstrable Wallerian degeneration. This time interval was determined by preliminary experiments. The frogs were sacrificed at various intervals of time ranging between 14 and 21 days. A careful study of the degeneration of the nerve fibers in serial section was done on the labyrinths of six frogs, stained by the method of Rasmussen. The labyrinth of another frog was studied in celloidin sections stained with several standard methods. The remaining seven frogs of the survival group were removed from the experiment after preliminary histological examination, because they did not meet the rigorous criteria established for the study. The nerves in these animals were transected in close proximity to the ganglions, and it could be seen with phase microscopy that in these animals the ganglion cells showed pathological changes.

In the animals selected, the surgical sections of the nerve were located close to the brain stem and more than 1 mm from the nearest sensory ganglion cell. In these animals no indication of degeneration in the cell body or in the fibers arriving at or leaving the ganglion cell could be detected. Fig. 1 is a photomicrograph of the VIIIth nerve ganglion and inner ear from the animal that was processed in celloidin sections. The results of this study are in agreement with the observations made by previous investigators that section of the VIIIth nerve does not produce degeneration of the ganglion cells of the vestibular end-organs (Wittmaack,

Recovery from anesthesia was enhanced by allowing a gentle stream of cool water to flow around the frog. When the frog awoke the functional effects of sectioning the VIIIth nerve were observed to be similar to those described by McNally & Tait (1925). The head was turned in a vertical plane relative to the body with the operated (right) side down. There was a tendency for the frog to lean toward the operated side and to extend the contralateral limbs.

The frogs were sacrificed after survival times varying between two and three weeks. Each frog was anesthetized deeply in the Tricaine solution, placed on its back on a dissection board and prepared for systemic perfusion by opening the chest and exposing the heart through the pericardial membrane. The blood was washed out of the circulatory system by perfusing it with 80 to 100 cc of frog Ringer solution under light pressure from a syringe. The tissues were then fixed by perfusing with 80 to 100 cc of 10% formalin. The head was removed and stripped of excess tissue and stored in 10% formalin until dissection of the labyrinth. Approximately one week later the membranous labyrinth and attached nerve trunk were dissected from the bone using a drill and forceps with the aid of a dissecting microscope. In this step the semi-circular canals were sometimes cut to facilitate removal of the labyrinth from the otic capsule.

The specimen was stained in bulk with Sudan Black B according to a modification of the method described by Rasmussen (1961). Thereafter the specimen was embedded in gelatin which was trimmed and mounted for cutting on the sliding microtome. The block was frozen using carbon dioxide. The labyrinth was placed ventral side up and oriented so that the anterior ramus of the VIIIth nerve could be sectioned longitudinally. The microtome was set for a section thickness of 15 to 18 microns through the longitudinal portion of the nerve. The remainder of the specimen was cut at 60 microns thickness. When the specimen was oriented properly the sensory organs of the two vertical semi-circular canals, the pars basilaris, and the pars amphibiorum were not reached by the sectioning knife until after the anterior ramus was completely sectioned. Therefore, these sensory areas were studied with sections thicker than those of the lagena, utriculus, sacculus and horizontal semi-circular canal.

In one of the operated frogs the whole head was decalcified, embedded in celloidin and sectioned transversally.

In the course of this study several ears from non-operated animals were processed and stained by different methods in order to become more familiar with the anatomy of the bullfrog's ear.

RESULTS

The surgical section of the VIIIth nerve was performed in 20 adult bullfrogs (rana catesbeiana). Fourteen frogs survived after the operation for more than two weeks, a time interval sufficient for the establishment

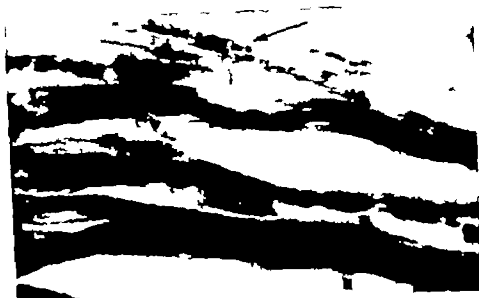


FIG. 3. The bundle of degenerated efferent fibers in the nerv to the utriculus.

ramus was cut transversally A detailed study of the fibers that form the post rior ramus could not be made until they reached the region of the sensory nd-organs.

Fibers to the End-Organs

At the level of the end-organs, the arri ing nerve fibers spread and bend to reach the various areas of the sensory epithelium Many of the fibers become quite separated from each othe and therefore more accessible to study A clear inspection of degenerated fibers could be made at this level The degenerated fibers observed were of small size, measuring from three to six microns in diameter (Fig. 4)

The number f degenerated fibers clearly identified in each one of the animals studied is summarized in Table I The results have been classified to show the number of efferent fibers demonstrated at the level of each f the sensory end-organs of the inner ear

The anterior ampullae of frog A2 and A6 were mutilated during prepara tion of the specimens, and search for degenerated fibers could not be made The same problem occurred with the posterior ampulla, the pars amphibiorum and the pars basilaris of frog A6

The number of degenerated fibers to the utricle in f og J1 was consider ably higher than in the other frogs. A possible explanation could be that the orient tion of this specimen on the microtome was such that a great portion of the utricular macula was cut in 60 micron sections, the preferred thickness for observing degenerated fibers in the sensory areas.

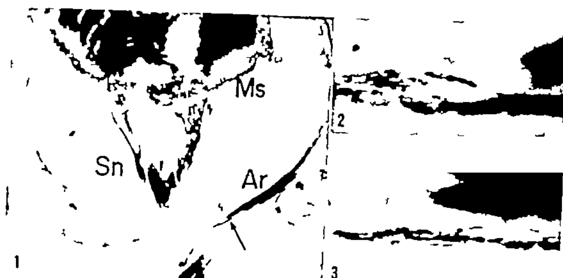


FIG. 2 Composite photomicrographs showing the degenerated fibers running al ng the anteri r ramus. (1) Low powe view showing the anterior ramu (Ar) and Macula of th Sacculus (Ms) and Saccular Nerve (Sn) The bundle f eff rent fibers run ing i the anterior ramus (arrow) (2) and (3) High power views of the area indicated by the arrow in the l w power view Each photograph c rresponds t different depth of focu at the same point.

1911 Kaida 1931 Hallpike & Rawdon Smith, 1934 Rasmussen & Gacek, 1958 Gacek, 1960 Ireland & Farkashidy 1961) In the present study the ganglion cells of the acoustic end-organs were without degeneration This is in disagreement with the observations reported in mammals (Schuknecht & Woellner 1953 Gacek 1960 Fisch & Ruben, 1962 Kimura & Wersall, 1962 Ruben Hudson & Chiong 1962 and Spoendlin & Gacek, 1963)

The Anterior Ramus

In the seven frogs studied a bundle of degenerated fibers was observed running along the anterior ramus These fibers are illustrated in Fig 2 These degenerated fibers join the nerve trunk coming from the postero-inferior side of the ganglion of the anterior ramus They were located on the ventral side of the nerve and could be followed running together as a bundle almost to the level of the utricular end-organ (Fig 3) It was difficult to determine the exact number of nerve fibers because of their compactness It was estimated that the bundle was made up of no less than six or seven but not more than twelve to fifteen fibers The approximate diameter of the visualized fibers is three to six microns.

The Posterior Ramus

The posterior ramus separates from the main trunk obliquely to the anterior ramus, thus, because of the chosen plane of sectioning, the posterior



Fig. 5 Photomicrograph showing degenerated fferent fiber t the lev l of the pars amphibiorum

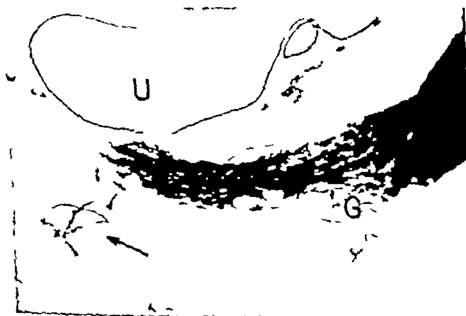


Fig. 6 Low power iew showing the nterior branch of the VIIIth nerv with its ganglion (G the utricl (U), and tangential section f the wall of the saccul where some nerv fibers were stained (arrow)



FIG. 4 Ph tomlerograph of a degenerated efferent fiber going toward the cupula of the lateral semicircula canal

Degenerated fibers were found going to the sensory epithelium of all the vestibular end-organs In the two generally accepted acoustic end-organs, the pars basilaris and pars amphibiorum degenerated fibers could be found only in the nerve going to the pars amphibiorum (Fig 5) These degenerated fibers were seen in three animals, A2 J1 and J10 In none of the animals was there any degeneration of the fibers going to the pars basilaris.

TABLE 1 *Number of degenerated fibers to each end-organ in six frogs*

Frog No	Survival time (days)	Sacculus	Utricle	Ampullae: Lateral S.C.	Ampullae: Ant r r S.C.	Ampullae: Posterio S.C.	Lagena	Pars amphi biorum	Pars basilaris
A5	14	6	3	5	4	9	6	0	0
A2	15	7	2	5	(*)	2	2	5	0
J1	15	4	12	7	5	4	5	4	0
J9	16	2	5	5	1	6	2	0	0
J10	16	4	2	3	2	5	2	5	0
J4	21	3	4	6	(*)	(*)	2	(*)	(*)

Search f r degenerated fibers could not be mad

This eliminated fibers that were not completely visualized because of the relative thickness of the sections, fibers that were cut obliquely fibers in which degeneration was not in a sufficiently advanced state, and possibly others in very advanced degenerative states in which the myelin droplets were already phagocyted It is also possible that there were degenerated fibers of caliber too small to be visible using the light microscope. All of the end-organs serving a vestibular function receive efferent fibers, as evidenced by the presence of degeneration while in the acoustic end-organs degenerated fibers were seen only in the pars amphibiorum

The anatomical demonstration of the existence of an efferent innervation of the vestibular apparatus of the frog confirms the earlier experimental physiological observations of Schmidt (1963 1965) and Gleisner & Hen rikason (1963) In his later work Schmidt (1965) has removed the doubts he originally had about the existence of efferent fibers to the pars amphi biorum. In the other acoustic end-organ, the pars ba ilaris, he has demon strated efferent nerve activity on only one occasion

Frishkopf & Geisler (1966) have been able to demonstrate the inhibition of primary auditory units in the VIIIth nerve of the bullfrog by the presence of other acoustic stimuli. The latency of the inhibitory phenomena is too brief to be explained as a consequence of a neural feed-back mechanism similar to the one existing in mammals. Therefore no evidence exists yet of the functional significance of the efferent fibers in the pars amphi biorum f the bullfrog's inner ear The existence of efferent nerve fibers innervating this end-organ a demonstrated in these experiments is sup-ported by the experiments of Flock & Flock (1966) which demonstrated the presence of efferent nerve endings at the base of the hair cells.

In mammal the different behavior of the cells f the Scarpa and Corti ganglions after transection of the VIIIth nerve between the brain stem and the sensory ganglion i the puzzle that has attracted attention of many investigators. In the frog the sensory ganglion and nerve fibers of the VIIIth nerve do not degenerate after sectioning the nerve between the brain stem and the sensory ganglion Their conduct f llows the rules of Wallerian degeneration. By contrast, in mammals there has been reported selecti e degeneration f the auditory ganglion cells and nerve fibers.

ACKNOWLEDGMENT

The uthors have th pleasure f xpressing thei recognition to Dr I H Ward for hi support nd encouragement during the investigation and h lp in the preparation of the man script.

ZUSAMMENFASSUNG

Der VIII. Gehirnnerv ines Frosches wurde zwischen dem Hirnstamm u d dem Si nesga glion chirurgisch durchtren t Zw i bi drei Wochen nach dem Ner enschnitt wurden die Frösch getötet und di W llerianische Entartungsreaktion



FIG. 7 High power lew of the nerve fibers the wall of the sacculu seen in Fig 6. Among the norm l fibers there are fibers in ad nced st tes of deg neration.

Fibers on Membranous Wall of the Sacculus

On the wall of the sacculus of each of the animals, fibers in groups of three to five were observed, distributed in a scattered fashion The origin of these fibers is undetermined and although their course suggests they are going to the macula of the sacculus, their destination cannot be positively stated Degeneration of some of these fibers was observed in all the frogs studied The existence of these degenerated fibers was of particular interest since they have not been described in any of the bibliographical references consulted in this study These fibers are most evident in the sections which had been cut tangentially to the wall of the ventral side of the sacculus. These fibers and the fibers of the anterior ramus are shown in Fig 6 A higher power view of these fibers shows some of them degenerated (Fig 7) These fibers could also be identified in transverse sections of the saccular wall using the Sudan Black staining method

DISCUSSION

The number of degenerated fibers shown in Table 1 are considered quite conservative since rigid criteria were followed in determining whether or not degeneration was present The actual number of degenerated fibers was probably larger than those reported because not all the fibers of the VIIIth nerve were visualized and accessible to study and only those fibers in which there was no doubt of the presence of degeneration were counted.

IRELAND, P W and F [illegible] J 1961 Studies on the efferent i nerv tion f the estibular end organ *Ann Otol* 70 490

I [illegible], S., 1962 Efferent fibers t the sensory cell of Corti' organ. *Exp. Cell Res* 27 162.

KAIDA, L 1931 Über da Verhalten des inneren Ohres, nach Stammlä ion des N. consti cus. *Jap. J Med Sci* Series 12, *Oto-rhino Laryng* 1 237

KR [illegible], R., nd W[illegible], J 1962) Terminati f the oli -cochlea bundle i rel tion t the outer hair cells f the organ f Corti i the guinea pig. *Act Otolaryng* (Stockh.) 55 11

K [illegible], 1880 Über das häutige Labyrinth der Amphibien *Arch. Mikrosk Anat* 17 479

McNALLY, W J and TAIT J 1925 Abl tion experiments on the l byri th f the frog *Ame J Physiol* 75 155.

PETROFF A. E., 1955 An experimental i estigatio of the origi f efferent fiber projection t the estibular neuro-epithelium *Anat Rec* 121 352.

PORTMA N, G PORTMANN, M nd PORTM NN, C., 1953 La double i nervation de l'org ne de Corti. *A ta Otolaryng* (Stockh.) 43 226.

RANSON, S. W 1903 Retrograde degeneration in the pinal nerves. *J Comp. Neurol A Psych.*, 10 1

RASMUSSEN, G L., 1946 The ll ry ped ncle nd ther fiber projections f the superior li ary complex. *J Comp. Neurol* 84 141

— 1953 Further observ ti ns of the fferent cochlea bundle. *J Comp Neurol* 99 61

— 1960 Efferent fibers of the cochlea nerv nd cochlea ncleus. I G. L. Rasmussen nd W F Windle *Neural Mechanisms of th Auditory and Vestibular Syst m* Charles C. Thomas, Springf ld, Ill., p. 105.

— 1961 A method f staining the statoacou tic nerv i bulk with Suda Black B. *Anat. Rec.*, 139 485.

RASMUSSEN, G L., nd GACEK, R R., 1958 Concerning the question f an efferent fiber component of the estibular nerv of the cat. *Anat Rec.*, 130 361

RETZIU s, G 1881 *Das Gehörorgan der Wirbelthi re* V I. I. Samson nd W llin, Stockholm.

ROSSI, G 1961 L Acetylcholinesterase cours d dév loppement d l'oreill interne du cobay *Act Ol laryng* (Stockh.) Suppl. 170

ROSSI, G and CORTESINA, G 1962 Il "sistema efferente colinergico cocleo-vestibolare S ntesi storico-bibliografica ricerche personali. *M nerva Ot rinolaring* 12, 173.

— 1965 The "efferent cochlea and estibular system in Lepus Cuniculus L. *Act Anat* 60, 362.

R [illegible], R. J HERMAN, W nd CHIONO, A 1962 Anatomical d physiological effects of chronic section of the ighth nerv i cat. *A t Otolaryng* (Stockh.) 55 473.

SCH [illegible] R S., 1963 Frog labyri thine efferent impulses. *Acta Otolaryng.* (Stockh.) 56 51

— 1965 Amphibia acoustico-lateralis efferents. *J Cell Comp. Physiol* 65 155

SCHU KNECHT H. F nd WOELLNER, R C., 1953 Hearing losses f llowing partial section of the cochlea nerv *Laryngoscope* 63 441

SCHU KNECHT H F CHURCHILL, J A., nd DORAN R., 1959 The localization f cetylcholinesterase i the cochlea *Arch Otolaryng* (Chic.) 69 549

S ITH, C. A., nd DEMPSEY, E. W 1957 Electron microscopy f the organ of Corti. *Ame J Anat* 100 337

S ITH, C. A., nd SJÖSTRAND, F S., 1961 Struct re of nerv endings of the external hai cell as studied by serial section. *J Ultrastruct Res.*, 5 523.

S ITH, C. A nd RASMUSSEN, G. L., 1963 Recent observ tions on the ll o-cochlear bundle *A Otol.*, 72, 489

— 1965 Degeneration i the efferent nerv endi gs i the cochlea fter onal section. *J Cell Biology* 26, 63

im Nerv wurde studiert. Es war möglich die Anwesenheit von zum Sinnesepithel verlaufenden efferenten Fasern aller anerkannten Vorhofendorgane und auch in Richtung der Papilla des Pars amphibiorum zu beweisen. Jedoch waren keine efferenten Fasern auf der Ebene der Papilla basilaris sichtbar. Die Ganglionzellen der akustischen und Vorhof zuführenden Fasern erschienen normal. Um die Sacculuswand waren viele ausgeartete Fasern in einer bis jetzt unbeschriebenen Gruppe von Fasern sichtbar.

REFERENCES

BOORD, R. L. 1961. The efferent cochlear bundle in the caiman and pigeon. *Exp. Neurol.* *3*: 225.

DESMEDT, J. E., 1960. Neurophysiologic l mechanisms controlli g acoustic input. In G. L. Rasmu sen and W. F. Windle: *Neural Mechanisms f the Auditory and Vestibular Systems.* Charles C. Th m s, Springfi ld, Ill. p. 152.

DESMEDT, J. E., and LAGRUTTA, V. 1963. Functi n of the uncrossed efferent livo-cochlear fibers in the cat. *Nature* *4905*: 472.

DOHLMAN, G., FARKASHIDY, J. and SALONNA, F. 1958. Centrifug l nerve-fibers t the sensory epithelium of the vestibula labyrinth. *J. Laryng.* *2*: 984.

ENGSTRÖM, H., 1958. On the d uble innervation of the sensory epithelia of the inner ear. *Acta Otolaryng.* (Stockh.) *49*: 109.

ENGSTRÖM, H. and WERSÄLL, J. 1958: The ultrastructural orga izati n f the organ of Corti and th vestibular sen ory epith li. *Exp. Cell Res.* *5*: 460.

FERNÁNDEZ, C., 1951: The inner tion f the cochlea (guinea pig). *Laryngoscope* *61*: 1152.

FEX, J. 1962: Auditory ctivity in centrifugal and centripetal cochlear fibers in cat. *A ta Physiol. Scand.*, Suppl. *189*: 1.

FISCH, U. and RUBEN, R. J. 1962: Electrical acoustical response to cli k stimulation after section of the ighth nerve. *Acta Otolaryng.* (Stockh.) *54*: 532.

FLOCK, A. a d FLOCK, B., 1966. Ultrastru ture f th amphibian papilla in the bullfrog. *J. Acou t. Soc. Ame.* *40*: 1262.

FRISHKOPF, L. S. d GEISLER, C. D. 1966. Peripheral origin of uditory respo ses recorded from the ighth nerve of the bullfrog. *J. A oust. Soc. Amer.* *40*: 469.

FRISHKOPF, L. S. and GOLDSTEIN, M. H. JR., 1963. Re ponses to co ti timuli from ingl units in the ighth nerve of the bullfrog. *J. A oust. Soc. Amer.* *35*: 1219.

GACEK, R. R. 1960. Eff rent component of vestibul r nerve. In G. L. R smussen and W. F. Wi dle: *N ral M chani m f the Auditory and Vestibular Systems.* Charles C. Th mas, Springfi ld, Ill. p. 276.

— 1961: The efferent cochlear bundle in ma . *Arch. Ot laryng.* (Chic.) *74*: 690.

GACEK, R. R. NOMURA, Y. and BALOGH, K., 1965. Acetyl h linesterase acti ity in the fferent fibers f th stato-acou tic n rve. *A ta Otol ryng.* (Stockh.) *59*: 541.

GALAMBOS, R. 1956. Suppressi n of a ditory n rv ctivity by timulati n of fferent fibers to cochl . *J. Neurophy i l.* *19*: 424.

GEISLER, D. C., BERGEIJK, W. A. v N. d FRISHKOPF, L. S. 1964. Th i er a of the bullfrog. *J. Mo ph.* *114*: 43.

GLEISNER, L., nd HENRIKSSON, N. G. 1963. Efferent and ffere t cti ity pattern i the ve tibular nerv f the frog. *A ta Ot. larg g.* (Stockh.) *192*: 90.

HALLPIKE, C. S. and R WDON SMITH, A. F. 1934. "Th Wever a d Bray Phenomena. A study of the electrical re po se in the cochlea with peci l ref rence to it rigi . *J. Physiol.* *81*: 395.

HILDING, D. nd WERSÄLL, J. 1962. Cholinesterase and it relat t the nerv endings in the inner ear. *Acta Otolary g.* (Stockh.) *55*: 205.

Acta oto-laryngologica 64, 440–443 (1967)

STUDIES ON THE PROTEIN IN CAT'S PERILYMPH AND THE EFFECT OF ULTRASOUND ON THE PROTEIN

H. C. LAWLER, J. G. WALTNER and M. BASEK
New York, N.Y., U.S.A.

From the Departments of Otolaryngology and Biochemistry, College of Physicians and Surgeons, Columbia University, New York

Perilymph samples were collected from normal cat and from cats subjected t ultrasoni irradiation and were nalyzed for protein. The control values were between 144.8 mg/100 g and 211.9 mg 100 g with a confidence interval of 95 per cent. Three days after irradiation the prot in concentration on the treated sid had increased significantly and then, in a week, it declined and th protein values obtained from both sides were similar and greater than the control values. It seemed that both ultrasound and stress produced n increase in permeability t serum proteins and the former caused greater increase. A few perilymph samples were collected from cases with Menière's disease. Serum subjected to ultrasound was examined by immunoelectrophoresis. The results were discussed in terms of th effect of ultrasound in the control of Menière's disease.

INTRODUCTION

As a result of the use of ultrasound in the surgery of the inner ear for the treatment of Menière's disease a number of histological and biochemical studies have been made of the changes produced (Sjöberg, 1964). While ultrasound controls vertigo in over 80% of the patients it is hoped that the optimum benefits follow from the effect on the secretion of labyrinthine fluids and microcirculation of blood. If biochemical alterations could be found the treatment might be modified to cause only the biochemical changes without destroying the vestibul sensory organs.

The effects of ultrasonics and its action on tissues have been studied extensively (Hughes & Nyborg, 1962; Graber, 1953; Hughes & Chou, 1964). In estigations ha e been made on the best surgical techniques to use in the treatment of Menière's disease (Waltner, 1965) and improvements have been made in the instrumentation (Basek & Epanchin, 1964). The information obtained from these studies has made it possibl to examine the chemical changes following the best operative procedures.

Th source and composition of the labyrinthine fluids has been studied xtensiv ly. Perilymph fluid i postulated by Hughes & Chou (1964) to be

Thi work was supported by the Division f Research Grant and F llow hips, U.S. Public Health Service, Grant No. NB — 2834.

Spoendlin, H. H. and Gacek, R. R. 1963. Electronmicroscopic study of the efferent and afferent innervation of the organ of Corti in the cat. *Ann Otol 72*: 660.
Wittmaack, K., 1911. Über sekundäre Degeneration im inneren Ohr nach Akustikusstamm-verletzungen. *Verh Deutsch Otol Ges 20*: 289.

Dept. of Otolaryngology, Vanderbilt University School of Medicine, Nashville, Tenn., U.S.A.

Received April 4, 1967

(1956) Solutions of Versatol were used as a standard in order to determine the conversion factor A factor of 146 was determined for protein concentrations of 12 μg per ml or higher and a smaller factor for lower concentrations. The difference in the absorption at 215 mμ and 225 m was multiplied by the conversion factor to give the protein concentration in the diluted solution in micrograms per ml When the absorption at 215 mμ exceeded 1 0 the absorption at 260 m and 280 m was determined and the protein concentration calculated from these values (Warburg & Christian, 1941) The samples were diluted further and read again at the lower wav lengths. Agreement was found in the values obtained by the two different spectrophotometric methods.

In some samples absorption in the region 320–360 m was found The absorption in this region might arise from pigment adsorbed to the protein or from haze due to scattered radiation (Beaven & Holiday 1952) A line from the absorption at 340 m and 350 mμ was projected as far as 260 m and served as a base line from which the optical densities at 280 m and 260 m were measured Samples which contained blood were discarded but it was found that in some apparently clear samples hemoglobin could be detected by the spectrophotometric method. The hemoglobin absorption at 410 m was determined and was corrected for the non-specific absorption at 340 m in the following way

O D (at 410 m corrected) = 4 0 (O.D 410) − 3.08 (O D 340)/3.23

As the optical density at 410 m had been determined for a known hemoglobin sample at a series of dilutions the related protein concentration value could be found from the optical density value

It was considered unlikely that the anesthetic, pentobarbital, would introduce an error in the protein determinations. If it was assumed that the pentobarbital appeared in the perilymph at a level comparable to the level in the plasma and cerebrospinal fluid estimated from the dose given (Brodie *et al.*, 1953) then the amount present in the sample collected would not be great enough to significantly increase the absorption

Samples of blood were collected from the heart and samples of cerebrospinal fluid from the subarachnoid space To examine the effects of ultrasound *on the* serum proteins, 0.2 ml of serum was placed in the well of a glass slide which was supported on a covered ice tray The Dallon's generator and a special transducer which has been described (Basek & Epanchin, 1964) wa adjusted to yield a total intensity output of 0.3 watts. It wa applied to the serum for the same period of time, for three two minute interv ls, chosen for the irradiation of the lateral semicircular canal

Immunoelectrophoretic examination of the proteins was undertaken by a modification of the micro-immunoelectrophoresis method described by

General Diagnostics Division, Warner-Chilcott Laboratory S pply Di ision, Morris Plains, New Jersey

slowly replaced by cerebrospinal fluid Altmann & Waltner (1950) did not find an active flow through the cochlear aqueduct unless a pressure gradient was established and Waltner (1948) demonstrated a barrier membrane at the tympanic end of the cochlear aqueduct In addition, other studies have indicated that perilymph is not a dialysate of the cerebrospinal fluid but is a blood filtrate (Vosteen 1961) The proposal is made on the basis of isotope studies and on the fact that the protein concentration is higher in the perilymph than in the cerebrospinal fluid Immunoelectrophoretic studies made by Chevance *et al* (1960 1964) indicated that serum proteins not present in the cerebrospinal fluid could be found in the perilymph Quantitative protein determinations on perilymph samples have been made by a number of investigators who have reported values of 142 mg/100 ml 208 mg/100 ml and even higher values (Waltner 1954 Citron, Exley & Hallpike, 1956 Ledoux, 1950)

Studies on the labyrinthine fluids were undertaken The chemical composition of the endolymph will be reported at another time (Lawler & Waltner)

METHODS

In the surgical treatment used the left lateral semicircular canal and ampulla were exposed In many cases the incus and a portion of the facial nerve were cut out and if the blue line could not be seen the bone was thinned with a dental burr Kossoff's ultrasonic generator was used The probe was applied to the horizontal canal near the ampulla or to the blue line of the ampulla

The ultrasound was administered at a total intensity of 0.33 watts for six minutes at two minute intervals with a pause of one minute between intervals During the irradiation the "irritative" nystagmus was followed and at the end the response to cold saline was observed On the few occasions when the response was not lost a few more minutes of irradiation was sufficient to destroy it Intramuscular penicillin was injected and injections were made at intervals until the perilymph samples were collected Samples from both ears were taken three days seven days or fourteen days after irradiation

Perilymph samples were collected from cats anesthetized with pentobarbital by a modification of the surgical technic described by Waltner (1954) A melting point capillary drawn down to a very narrow tip was inserted through the dry round window membrane and allowed to remain for fifteen seconds The sample collected was transferred to a tared stoppered weighing vessel and weighed immediately on a Mettler microbalance It was diluted approximately a hundred fold by the addition of 0 4 ml of 2% $(NH_4)_2SO_4$ adjusted to pH 6 1 and the absorption determined against a blank containing the diluent in the Zeiss PMQ spectrophotometer

The protein concentration was determined by the ultraviolet spectrophotometric method described by Murphy & Kies (1960) and Waddell

proportional to the concentration and to manifest itself as a constant coefficient of variation which it did The right skewness and a constant coefficient of variation necessitated a logarithmic transformation to achieve homoskedasticity and normality Bartlett s test accepted the proposition of homoskedasticity in logarithmic concentrations. Analysis was performed within the transformation Since the analysis is relatively more robust towards anormality than inequality of variance it was felt that the parametric prerequisites were reasonably satisfied.

Type I error of which 0.05 was chosen as the critical value was controlled at the level of four sub-experiments. The first sub-experiment established a confidence interval for the protein concentration in the control group the second, compared the values obtained from the unoperated ear after irradiation of the ampulla and the horizontal canal with each other and with the control values the third and fourth sub-experiments on the samples obtained after irradiation of the ampulla and the horizontal canal respectively compared the differences between the treated and control groups in time and the differences between the treated and untreated ears three days after treatment It must be stated that the hypotheses were formulated *a priori*. In the third and fourth sub-experiment an incomplete three way analysis of variance was utilized for testing within each treatment group and the time-treatment interaction mean square served as the error term In comparisons involving the effect of treatment on the unoperated sides between the ampulla and horizontal canal groups and these groups and the control groups a pooled within mean square of the comparison columns was calculated and served as the error term in *t*-testing

RESULTS

The protein values in perilymph collected from control and treated cats are reported in Tables 1 2, and 3. The range of values are quite large which was to be expected as no attempt was made to limit the variables within the cat population due to differences in weights, age sex, health nd general condition of the animal

The observed distribution hown in Fig. 1 was based on a sample size f 46 from a population which consisted of control samples, samples from cats which had been operated upon but had not been irradiated (sham") and samples from the untreated ear three days after irradiation of the ampulla. The samples below log 1 9 were considered to be significantly cont m nated with cerebrospinal fluid This may occur if excessive amounts of per lymph ar removed by aspiration which ruptures the barrier membrane They were not included in the analysis. The distribution of the control samples was found t fall under the main peak whereas the distribution of the sham samples fell mainly under the second peak The pattern s ggested that there might be an increase in protein concentration that wa not directly due t irradiation. Unfortunately there were not

Scheidegger (1955) To each of six slides, 76 by 26 mm, was added 3 ml of 1 per cent Ionagar No 2 in 1.5×10^{-3} M calcium lactate and 2×10^{-2} M veronal buffer at pH 8.35 In each buffer compartment of the Buchler micro chamber was placed a solution of 5×10^{-2} M veronal buffer pH 8.35, 1.25×10^{-3} M calcium lactate, and 0 1 per cent sodium azide. The electrophoretic separation was carried out at 5 and 200 volts for 150 minutes. Then some of the slides were stained with Light Green stain to reveal the separation of the proteins and to the other slides was added 0 1 ml of the immune serum[1] The slides were kept at 5 for five days to allow the immunoelectrophoretic pattern to develop

An examination of the composition and handling of the perilymph samples revealed a number of possible sources of error Therefore protein values were eliminated from the statistical analysis which might be questioned for any of the following reasons (1) evaporation during weighing (2) evaporation during prolonged storage (3) contamination by blood (4) massive contamination by cerebrospinal fluid Although the samples were weighed rapidly it was thought that a slight evaporation might affect the weight of samples of less than 1 mg significantly and they were not included If the samples collected were not analyzed immediately they were drawn up into the capillary with gentle suction and stored at − 10 After forty-eight hours a loss in weight due to evaporation could be detected and therefore samples stored for longer periods of time were not included A correction for the blood contamination was made which was based on the determined hemoglobin concentration and an assumed average value for the amount of hemoglobin in blood Because of the assumption any sample was rejected that had an appreciable amount of blood as judged by the criterion of an optical density at 410 mμ for the diluted sample greater than 0 025 and larger than the optical density at 340 mμ The contamination by cerebrospinal fluid will be discussed

Statistical Considerations

In order to apply the parametric technics of analysis to the data it was necessary to satisfy the criteria of normality and homoskedasticity of the treatment variable amongst the various treatment groups. The characteristics of the distribution were not known but could be inferred from the following considerations Protein distributions in body fluids range from normality to a certain degree of positive skewness as lower boundaries on concentration are more relatively fixed in health than are the upper boundaries. The empirical distribution of the data mildly suggests it but there are not enough samples to confirm it In addition, there was a possible error which resulted from the mixing of perilymph with cerebrospinal fluid during the sampling The error was expected to be inversely

[1] Rabbit anti-cat serum goat anti-human serum, lot no 3802A8, from Hyland Laboratories, Los Angeles, California

TABLE 2. *Protein concentration in perilymph samples collected at the indicated times following irradiation of the ampulla.*

Values in mg protein/100 g perilymph re reported from both ears of each cat.

Three day		Seven days		Fourteen days	
Treated	Untreated	Treated	Untreated	Treated	Untreated
96.6[a]	58.–[a]	42.5[a]	229.3	96.1	110.5
202.9	253.0	76.1	114.5	137.7	223.7
331.0[a]		100.7	330.4	154.1	
376.5	81.0[a]	182.7		182.7	240.0
376.0	228.8	167.3	190.2	175.6	181.6
384.0	519.8[a]	188.0	263.1	232.8	436.6
402.1	134.6	217.3[a]	133.8[a]	239.0	335.0
487.1	150.7	250.0	608.0	307.9	202.8
591.8	494.0	269.0	313.3	387.0	296.0
970.5	297.2	323.–[a]	174.5	437.0	461.0
2031.3	396.9	398.6[a]		470.5	547.0[a]
2213.0	156.6	400.5	427.8	4 6.7	143.0
2867.0	110.1	619.8	431.0		231.9
3710.0	748.0[a]	890.0[a]	363.3		
		1078.0[a]	365.2[a]		
		1132.3[a]	307.1[a]		
		1880.0	419.1		
			364.2[a]		

Values were not included in the statistical nalysis as they were considered t be in error for) one of the reasons discussed i the text.

tion of the unoperated side in the ampulla irradiated cats was equal to th a erage control value was tested by the *t* distribution method and rejected The null hypothesis that the average protein values in the un treated ear within three days to two weeks after treatment by irradiation of the ampulla are equal to the values in the untreated ear after irradia tion of the horizontal canal was tested in the same way and accepted Therefore it i inferred that the protein concentration on the unoperated side is increased indirectly above the control as a result of the ultrasound or f the operation or both Although less ultrasound would enter the labyrinth after it was applied to th horizontal canal than would enter from the ampulla the response can be f und in the unoperated ear after either treatment It is pos ible that the contralateral increase in protein is due to the trauma produced by the operation and may not be due to the ultrasound.

In order t demonstrate the effect of ultrasonic irradiation of the mpulla on the protein concentration of the perilymph at the different time intervals following treatment the mean log value of the protein concentra tion on the perated and unoperated sides were pl tted gainst time (Fig.

TABLE 1 *Protein concentration in perilymph (mg protein/100 g perilymph)*

Control		"Sham" Operated[b]		Lateral canal irradiated[c]	
		Operated	Unoperated	Irradiated	Untreated
59 1		00 6[a]	64.8[a]	110 0	210.3
72.3[a]	143.3	83 7[a]	121 7	132.0	155.3
94 6	103.3	183.8	311.9	204 6	206.4
		251.9	271.3	303.6	293.4
134 6	188.2	288 0	68 6[a]		335.0
		361 7	745 0[a]	653.0[a]	684 6
144.3	170.3	451 0	268.0		
150.5					
157 6	384.5				
109 0	160 1				
196.7	253.2				
223.2					
302.5	540.3[a]				
423.4					
456.0[a]					

In the three groups the samples in the left and right columns were collected from the same cat There is a blank when a sample could not be collected.

[a] Values were not included in the statistical analysis as they were considered to be in error for any one of the reasons discussed in the text

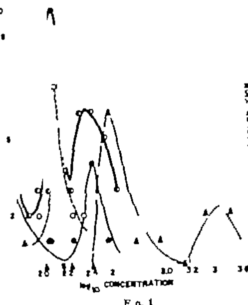
[b] The surgical procedure used on all the treated cats except that the ear was not irradiated

[c] The samples were collected within five hours following irradiation.

enough sham samples to analyze statistically Most of the samples collected from the untreated ear three days after irradiation were found to fall under the second peak and the possibility of a contralateral increase in protein was considered The distribution of the samples collected from the treated ear three days after irradiation showed two distributions and therefore the two groups were considered separately Protein values of 1000 mg/100 g or over were not used in the analysis as they distorted the distribution values and were considered to represent special "threshold" phenomena

In order to demonstrate changes in the protein concentration in the perilymph as a result of ultrasonic treatment it was necessary to find the normal range A confidence interval for the protein concentration was established with the use of the t distribution With 95 per cent confidence the true average protein concentration in the control cats is between 144 8 and 211 0 mg/100 g

An examination of the protein concentrations on the unoperated side after irradiation of the ampulla and after irradiation of the horizontal canal was made and the two groups were compared with each other and with the controls. The null hypothesis that the average protein concentra



F G. 1

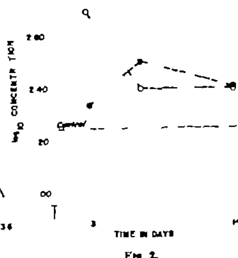


Fig. 2.

Fig. 1 Distributio curves f the protein concentration in perilymph (mg prot in/100 g perilymph) transformed t logarithms. O——O A population whi h represents samples collected from th ears of control cats, "sham operated cats and the untreated ear of cat which had been subjected to irradiation of the opposite ear three days prior t the collection of the samples. ○ ○ Samples from control cats. ●——● Samples from "sham perated cats. ▲ ▲ Samples collected from the treated ea three days aft irradiation.

Fig. 2. The mean logarithmic protein concentratio in the perilymph as functio of time followi g irradiation. The open circles represent samples collected from the irradiated ea and the losed circles represent samples collected from the ntreated ear The mean logarithmic control lue is recorded as straight lin Conce tratio is reported mg protein/100 g perilymph.

subjected to irradiation of the horizontal canal were analyzed in a similar fashion (Tabl 5) The proposition of equality of form and level between the treated and untreated ears was accepted within the framework of a similar analysis of ariance procedure Therefore the protein concentrations in the perilymph from th operated and unoperated sides, three days after irradiation must be considered to be similar

As a esult of the analysis it is apparent that ultrasonic irradiation of the ampulla caused an increase in the protein concentration in perilymph of the treated ear which reached its maximum in approximately three days and returned to the lev l of the concentration found on the unoperated ide within seven days. The values for the prot in concentration in the perilymph collected three days after irradiation are reported in Table 2. The values which exceeded 1000 mg/100 g were not included in the sta tistical analysis because they might represent either the appearance of

TABLE 3 *Protein concentration in perilymph samples collected at the indicated times following irradiation of the lateral canal.*

Values in mg protein/100 g perilymph are reported from both ears of each cat.

Three days		Seven days		Fourteen days	
Treated	Untreated	Treated	Untreated	Treated	Untreated
109.3	220.9	101 7	443.8	106.0	153.9[a]
208.2	162.3	121 0	269 9	131 7	102.9
260.5	216.3	150.5	397.5	132.0	291 7
276 0	500 0[a]	157 7	114 4	181 –[a]	163.0[a]
277 7	442.0	173 1	201 0	230.7	329.6[a]
291.8	344 0	228.0[a]	169 6	319.3	167.5
			87.8	319 7	303.3
			307 9	321 0	197.8
				354.3	209 1
				403.2	874.0
				415.0	
				417.5[a]	234.5
				504 6[a]	
				649.9[a]	438.5[a]
				792.5	115.5[a]
				1103.0	368.0

[a] Values were not included in the statistical analysis as they were considered to be in error for any one of the reasons discussed in the text.

2) From the height and trends of the averages it was possible to compare differences of the operated and unoperated sides with each other and with the control groups At seven days and fourteen days the values from the operated and unoperated sides were similar to each other and greater than the control values However after three days the protein values for the perilymph from the operated side were significantly higher than the values from the unoperated side The null hypothesis that there is an equality of form and level of response between the ampulla irradiated and the unoperated side simultaneously in time was examined by an F procedure in an incomplete three way analysis of variance and was rejected (Table 4) There is a signifant inequality in differences in levels or the way these differences vary in time or both It is evident (Fig 2) that the greatest difference appeared three days after the operation The perilymph samples obtained three days after the operation from both the operated and unoperated ears were paired in order to eliminate variations which would exist between the cats The null hypothesis that there was no difference between the two sides was analyzed by the t distribution method and rejected The protein concentration on the irradiated side was significantly higher than on the unoperated side three days after the operation

The protein concentration values obtained from the cats which had been

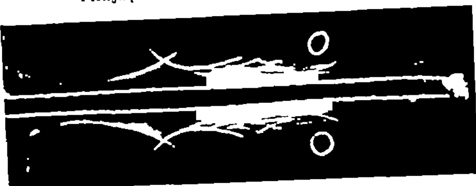


Fig. 3. Imm noelectrophoretic nal is f cat serum revealed by immune serum against normal cat serum. Below normal cat serum. Above normal cat serum which had been irradiated.

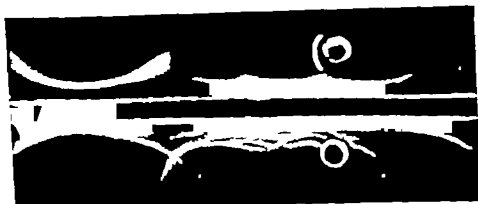


Fig. 4 Immunoelectrophoretic nal is f cerebrospinal fluid rev led by immune serum gainst normal cat serum Bel w normal cat serum Above, cat cerebrospinal fluid concentrated on Sephadex G-25.

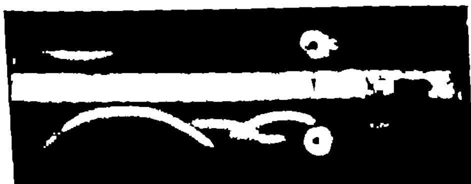


Fig. 5. Immunoelectrophoretic nalysis of huma perilymph revealed by goat immune serum against normal huma serum Below normal huma serum. Above, huma perilymph. Although the photograph reveals onl on precipitation lin three additional faint lines were found after the lide w stained.)

TABLE 4 *Incomplete three way analysis of variance*

Fixed mod l R nd m individuals Tr = treatment Id = individuals *t* = time

	df	SS	M.S.	$F_{(obs)}$	$F_{(t,m)}$
Tr (*t*)	3	0 34935	0 11651		
Tr	1	0 02100	0 02100		
Tr–*t*	2	0 32785	0.16128	6.301	3.57
Id (*t*)	19	1 08600	0.57189		
Tr–Id (*t*)	19	0 49510	0.26058		
CT (*t*)	3	259 7766			
CT	1	259 6601			
t	2	0 1165	0 03825		

protein from an additional source or indicate that a threshold had been passed which caused a higher flow of protein into the fluid

The statistical evidence for the increase in the protein concentration on the untreated side above the control levels was most interesting The higher values were maintained for more than two weeks following the operation The protein values on the operated side dropped to the level of the values on the untreated side seven days after irradiation of the ampulla The irradiation of the horizontal canal caused a similar increase in protein concentrations on both the treated and untreated sides. Recovery from the protein increase due to the nonspecific stimulation did not occur within the experimental period It will be discussed

The studies on the serum proteins which had been directly affected by ultrasound did not reveal any major changes in the proteins In Fig 3 the immunoelectrophoretic pattern of serum subjected to ultrasound is compared with normal serum at the same protein concentration Very minor changes can be seen in the irradiated serum The alterations in the precipitation curves for the a_2 macroglobulin the β lipoprotein another β-

TABLE 5 *Incomplete three way analysis of variance*

Fi ed mod l R nd m indi id ls. T = treatme t Id = indi idual *t* = time

	df	SS	M S	$F_{(obs)}$	$F_{(t,m)}$
Tr (*t*)	4	0.20853			
Tr	1	0 01411	0.01411		
Tr–*t*	3	0 19142	0.06181	2.29	3.20
Id (*t*)	17	0 03634	0.05308		
Tr–Id (*t*)	17	0 47918	0 02810		
CT (*t*)	4	230.76142			
CT	1	230.61727			
t	3	0.11415			

a temperature rise Mechanical effects increased permeability as was demonstrated by Hughes & Chou (1964) on isolated muscle Cavitation or the shear caused by the eddying action injures cells, degrades macromolecules and releases cell components (Hughes & Nyborg, 1962 Grabar 1953) Only slight changes in the macromolecules of the serum were made by the intensity of ultrasound used in these studies and it is improbable that such changes could affect the protein concentration However the indication that the serum became more alkaline should be explored further

The temperature increase was kept within safe limits by an application of ultrasound for periods of only two minutes (Basek & Epanchin, 1964) At the end of treatment, no eye movements could be elicited by the appli cation of cold saline solution to the ampulla which indicated either a complete destruction or temporary damage to the vestibular endorgan or vestibular nerve

Following irradiation, histological examination has revealed dilated blood vessels in the cristae of the semicircular canals and in the utricular and saccular maculae disorganization of the neuroepithelium and absence of the cuticular membrane (Dalton, 1964) After larger doses of ultrasound the membranous labyrinth was extensively damaged the posterior semicircular canal collapsed and was pushed to one side and the organ of Corti showed some degeneration (Formby 1963) The intensity of irradiation used in the present studies would be expected to affect the hair cells in the vestibular endorgans and possibly cause hemorrhage as was reported by Angell James *et al* (1963) It also might affect the vestibular nerve that consists of afferent and efferent fibers which f rm part of the pathway for the reciprocal innervation of the labyrinths on both sides (*Gleisner & Henriksson*, 1964)

At the present time Menière s disease is controlled by the ultrasonic destruction of the cristae of the lateral semi-circular canals (Waltner 1964) However the destruction may not be necessary if lower doses of ultrasound can act on the system affected by the disease The theory that the disease is due t a circulatory disorder has wide support Shambaugh has proposed that it is due to an imbalance of the autonomic system The evidence of n increased protein concentration in the perilymph probably derived from the blood would suggest that the ultrasound had affected the vascular ystem either directly or through its autonomic regulation and, therefore may be able to affect the system which produces the symptoms

Three d ys following irradiation of the ampulla the increase in protein in the perilymph collected from the untreated ear is of a smaller magnitude than the increase in the treated ear It is assumed to be a stress response and may be mediated either locally by a reciprocal innervation of the uton mic system or systemically by a release of adrenalin or histamine Kluyskens & Vantrappen (1961) and Chevance (1958) found modification and probable increase in the protein of the perilymph following the in jection f a non-specific irritant Ottaviani, Pesenti & Aliprandi (1963)

globulin and the γ-globulin were so slight that the ultrasound had not markedly changed the structure of the proteins Determination of the protein concentration of the irradiated serum by the ultraviolet spectrophotometric method showed a twenty per cent increase in protein over the untreated sample of serum The increase might be due to evaporation during the irradiation or to a slight unfolding of the protein structure which would bring more bonds into a position to adsorb Irradiated serum was found to adsorb more dye The pH of one sample of irradiated serum was determined and was found to be above 8 Therefore, ultrasound may affect directly the components in the perilymph fluid but the studies were not extensive enough to justify that conclusion

The immunoelectrophoretic patterns of cat cerebrospinal fluid Fig 4 and human perilymph Fig 5 are shown Cat cerebrospinal fluid formed four precipitation arcs and the albumin concentration seemed to be relatively higher than in the serum The immunoelectrophoretic pattern of human perilymph revealed only one precipitation arc which was attributed to albumin The mobility of the serum albumin was greater than the perilymph albumin As equal quantities of protein were added to each well the absence of proteins other than albumin in the perilymph immunoelectrophoretic picture might be attributed to a denaturation of perilymph proteins during the concentration procedure

A few samples of human perilymph were collected from subjects with Menière s disease The best sample contained 128.2 mg protein/100 g perilymph fluid which seemed to be slightly lower than the values in the normal range The other samples had values above the normal values but were questioned because there may have been evaporation of the samples.

DISCUSSION

The significant increase in the protein concentration of the perilymph on the treated side three days after irradiation of the ampulla might be attributed to degeneration of tissue proteins or to contamination with endolymph if the protein concentration of the endolymph had increased or to an elevated filtration of blood into the labyrinth It had been hoped that the proteins found in the perilymph could be characterized by immunoelectrophoresis but it was impossible to concentrate satisfactorily the samples which had been diluted for spectrophotometric analysis It would be reasonable to assume that the intensity of ultrasound administered was too small either to destroy enough tissue to account for the large increase in protein or to make lesions in the membranes which would permit a mixing of endolymph with perilymph It is believed that the additional proteins came from the blood as a result of an increase in permeability and the very high protein concentrations, over 1000 mg 100 g resulted from lesions in the vascular system

The vibrating waves of ultrasound produce mechanical effects and cause

Chevance, L. G., Galli, A., Jeanneret, J. and Gerard, G. 1964. Perilymphatic liquid. I. *Immunoelectrophoretic Analysis* (ed. P. Grabar and P. Burtin), p. 252. Elsevier Publishing Company, New York.

Citron, L., Exley, D. and Hallpike, C. S. 1956. Formation, circulation and chemical properties of the labyrinthine fluids. *Brit. Med. Bull.* 12, 101.

Dalton, G. A. 1964. Histological effect of ultrasound on the labyrinth of the sheep. *Acta Otolaryng.* (Stockh.) Suppl. 192, 183.

Fernet, M. L. 1963. Ultrasonic destruction of the labyrinth. *Acta Otolaryng.* (Stockh.) 56, 139.

Gleisner, L. and Henriksson, N. G. 1964. Efferent and afferent activity pattern in the vestibular nerve of the frog. *Acta Otolaryng.* (Stockh.) Suppl. 192, 90.

Graber, P. 1953. Biological actions of ultrasonic waves. *Adv. Biol. Med. Physics*, 3, 191.

Hughes, D. E. and Nyborg, W. L. 1962. Cell disruption by ultrasound. *Science* 138, 108.

Hughes, D. E. and Chou, J. T. Y. 1964. The biochemistry of the inner ear and the consequences of treatment by ultrasound. *Acta Otolaryng.* (Stockh.) Suppl. 192, 189.

Kluyskens, P. and Vanderkerckhove, W. 1961. Micro-électrophorèse de la périlymphe. *Acta Otorhinolaryng. Belg.* 15, 244.

Lawrence, R. C. and Waltner, J. G. (to be published).

Ledoux, A. 1950: Les liquides labyrinthiques. *Acta Otorhinolaryng. Belg.* 4, 216.

Maurer, J. B. and Kahn, M. W. 1960. Note on spectrophotometric determination of proteins in dilute solutions. *Biochem. Biophys. Acta*, 45, 352.

Otte, A., Pernetti, M. and Altmann, G. 1963. Immuno-electrophoretic study of the perilymph. *Acta Otolaryng.* (Stockh.) 56, 715.

Schreiber, J. J. 1955. L'immuno-électrophorèse. *Sem. Hôp. Paris* 31, 2119.

Sjöberg, A. 1964. Proc. Int. Vestibular Symp., Uppsala, 1963. *Acta Otolaryng.* (Stockh.) Suppl. 192.

Vosteen, K. H. 1961. Neue Aspekte zur Biologie und Pathologie des Innenohres. *Arch. Ohr. Nas. Kehlk.-Heilk.* 178, 1 (1963). Translations Beltone Inst. for Hearing Research, Chicago.

Waddell, W. J. 1956. A simple ultraviolet spectrophotometric method for the determination of protein. *J. Lab. Clin. Med.* 48, 311.

Waltner, J. G. 1948. Barrier membrane of the cochlear aqueduct. *Arch. Otolaryng. Chic.* 47, 656.

— 1954. The chemical composition of perilymph in cats. *Laryngoscope* 64, 439.

— 1964. Ultrasound in the treatment of Menière's disease. *Acta Otolaryng.* (Stockh.) Suppl. 192, 189.

— 1965. Ultrasonic surgery in Menière's disease. *Ann. Otol.* 74, 174.

Warburg, O. and Christian, W. 1941. Isolierung und Kristallisation des Gärungsferments Enolase. *Biochem. Z.* 310, 384.

*H. C. Lawrence, Ph.D. Depts. of Otolaryngology and Biochemistry
College of Physicians and Surgeons,
Columbia University, New York, N.Y. U.S.A.*

Received January 30, 1967

found that the administration of adrenalin or histamine caused the appearance of larger molecular weight serum protein in the perilymph. The duration of the response for one or two weeks after the irradiation indicates either a prolonged response to the initial operation or a sensitization that causes an immediate response when the ear is reopened to collect a sample. It is possible also that any form of stress will cause an increase in the perilymph protein concentrations which may continue throughout the recovery period. The increased protein values were also found in the so called sham operated cats which had been subjected to a surgical stress and therefore it is possible that some of the higher protein values reported in the literature may reflect a stress situation.

ACKNOWLEDGMENTS

The authors would like to thank Lyon Hyams M.D., M.S. for the extensive statistical analysis. The technical assistance of Miss Rasma Kalnins is gratefully acknowledged.

ZUSAMMENFASSUNG

Die bedeutungsvolle Zunahme des Proteingehaltes der Perilymphe 3 Tage nach Beschallung des lateralen Bogenganges mit Ultraschall ist höchstwahrscheinlich die Folge einer Zunahme der Permeabilität des Gefäss-Systems. Die Zunahme des Proteingehaltes auf der *unbehandelten* Seite war weniger ausgeprägt als auf der behandelten Seite. Sie stellt vermutlich eine „Stress" Reaktion dar, die entweder lokal infolge der reziproken Innervation des autonomen Nervensystems zustande kommt oder auf dem Wege des Kreislaufes. Möglicherweise verursacht jede beliebige Art von „Stress" eine Vermehrung des Proteingehaltes der Perilymphe, die während der ganzen Erholungsperiode fortbestehen kann.

REFERENCES

Altmann, F. and Waltner, J. G. 1950. Further investigations on the physiology of the labyrinthine fluid. *Ann Otol* 59 657.

Angell James, J., Dalton, G. A., Hadley, K. J., Freundlich, H. F., Bullen, M. A. and Wells, P. N. T. 1963. A new 3-megacycle generator for destruction of the vestibular end organ. *Acta Otolaryng* (Stockh.) 56 148.

Basek, M. and Edelbach, ... 1964. New ultrasonic ... for treating Meniere disease. *Arch Otolaryng* (Chic.) 80 271.

Beaven, G. H. and Holiday, E. R. 1952. Ultraviolet absorption spectra of proteins and amino acids. *Adv Prot Chem* 7 36.

Brodie, B. B., Burns, J. J., Mark, L. C., Lief, P. A., Bernstein, E. and Papper, E. M. 1953. The fate of pentobarbital in man and dog and a method for its estimation in biological material. *J Pharmacol Exp Ther* 109 26.

Chevance, L. G. 1958. Preliminary note on the biochemical aspect of the stimulation of the autonomic nervous system of the guinea pig cochlea. *Acta Otolaryng* (Stockh.) 49 309.

Chevance, L. G., Galli, A. and Jeanneret, J. 1960. Immuno-electrophoretic study of the human perilymph. *Acta Otolaryng* (Stockh.) 52 41.

TABLE 1 *Summary of the temperatures measured using different ultrasonic apparatuses in the vestibule cochlea and facial nerve in human temporal bone specimens at different ultrasonic powers*

The values are largely taken from published figures. All the authors irradiated the blue line on the lateral semicircular canal.

Author	Apparatus	Frequency (MHz)	Tip diam. (mm)	Average intensity (W/cm²)	Total power (W)	Recorded temperature (°C) Vestibule	Cochlea	Facial nerve
Basek & Epanchin (1964)	Federici	1	5		0.5	39		43
					0.75	46		58
Angell James (1963)	Bristol	3	2*	20	0.75	43	38	46
				30	1.1	46	30	50
Angell James *et al.* (1963)								
Basek & Epanchin (1964)	Dallon	1	3		0.5	43		48
					0.75	47		55
					1.0	48		57
Basek & Epanchin (1964)	Kossoff	3	2.6		0.5	38.5		40
					0.75	39		41
					1.0	39.5		42

* Radiating area 3.7 mm²

directed towards the blue line on the lateral semicircular canal and the total output power varied between 0.5 and 1.1 watts. The rise in temperature was consistently much greater in the facial nerve than in the vestibule. Studies on the temperature of the temporal bones of living animals treated with ultrasound have also been published (Angell James, 1963; Basek & Epanchin, 1964) but the results have often shown considerable differences from the results measured on human temporal bones (Angell James, 1963).

The question of whether the horizontal nystagmus which appears initially during ultrasonic treatment is calorically induced or not was analysed by Arslan, Sala & Molinari (1963) in rabbits. With the rabbit in different position Arslan and his co-workers observed the direction of the nystagmus during ultrasonic irradiation of the lateral semicircular canal. The direction changed in complete conformity with the endolymph currents, which can be expected from the heat supplied during the ultrasonic treatment. The application of a warm rod to the lateral semicircular canal with the rabbit in the different positions, produced the same direction of the nystagmus as ultrasonic treatment. Stahle & Sahl (1964) studied the same

Acta oto-laryngologica 64 464–476 (1967)

SOME THERMAL EFFECTS OF ULTRASOUND ON THE INNER EAR

B DRETTNER S. JOHNSON A SJÖBERG and J STAHLE

Uppsala Sweden

*From the Department of Otolaryngology (Head Prof A Sjöberg)
University Hospital Uppsala*

Temperature measurements on isolated human temporal bones in connection with ultrasonic irradiation showed that when the transducer was applied to the enchondral bone in the angle between the lateral and the superior vertical semicircular canals, the rise in temperature in the vestibule was as great as or greater than that in the facial nerve while the studies reported in the literature with the transducer applied to the blue line on the lateral semicircular canal constantly showed higher temperatures in the facial nerve than in the vestibule The rise in temperature in the cochlea was small Measurements of the temperature in the vestibule and in the lateral semicircular canal during ultrasonic irradiation of a patient with Menière s disease yielded roughly the same result at lower ultrasonic powers as the studies of isolated temporal bones, while at higher powers there was a smaller temperature rise *in vivo* than in the specimens.

Ultrasonic treatment of rabbits and human beings in different positions showed that the nystagmus which appears during irradiation is probably a caloric reaction induced by the thermal effect of the ultrasound

INTRODUCTION

Ultrasound is considered to have thermal mechanical and chemical effects on the tissues of the inner ear The facial palsy reported after ultrasonic treatment in Menière s disease has constantly been ascribed to too great an increase in the temperature of the nerve (Bullen *et al.*, 1963 Basek & Epanchin, 1964) The critical temperature of the facial nerve is considered to be about 46 C (Angell James *et al* 1964) In the sciatic nerve of the frog the action potential is reduced by 50% when the temperature reaches the 48–51 C range (Anderson *et al* 1951)

Several examinations of the effect of ultrasonic treatment on the temperature of isolated human temporal bones have been published in recent years A summary of the results with different ultrasonic apparatuses is shown in Table 1 In order to obtain a temperature of the specimen which was substantially the same as that *in vivo* the specimen was in most cases immersed in warm water In all the cases reported the transducer was

This work was supported by the Swedish Medical Research Council (Project No 17X-617-01K)

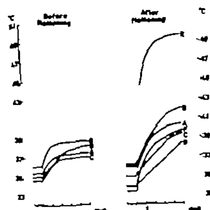


Fig. 1 The t mperatures in different part of temporal-bone specimen in connection with conti uous ultrasonic irradiation with power of 4 watt of the lateral semicircular canal before nd fter fl tteni g f the blue line The thermo-couple wa introduced through the al window I the vestibule (*A*) f rther back towards the labyrinth (*B*), forw rds in the direction of the cochlea (*C*) through the round window int the cochlea (*D*) I the lateral semicircula canal (*E*) or I the facial nerv immediately behind the ov l window (*F*) The specime w immersed in the surf ce layer of thermostatically controlled w ter-bath The transducer had metal tip (diameter 2.5 mm)

were then applied either in the operation cavity or in the inner ear In the latter case the measurements were made in connection with the surgical destruction of the labyrinth

RESULTS

Temperature studies on isolated temporal bones

The change of temperature during ultrasonic irradiation of the lateral semicircular canal in a specimen of a human temporal bone is shown in Fig. 1 The transducer had a metal tip with a diameter of 2.5 mm. The output power was 4 watts. Before the bone above the lateral semicircular canal wa thinned out, the rise in temperature in the vestibule and the cochlea was very moderate while a con iderably higher temperature was reached after the bone had been drilled d wn to the blue line In the basal part of the cochlea the temperature rose 4.5 C after 5 minutes continuous irradiation of the blue line In the vestibule the increase am unted to ~6 C and in the facial nerve behind the oval window to about 0 C. The largest increase was mea ured, a expected, in the lateral semicircular an l, where a temperature of 50 C was reached after 5 minutes irradiation

Fig 2 show the results f temperature measurements on another temporal-bon specimen, in which the same ultrasonic transducer as above wa pplied to the enchondral bone in the angle between the lateral and

problem in man by recording the nystagmus during the ultrasonic treatment of two patients suffering from Menière's disease, first with the face of the patient turned upwards and then with the face downwards. Neither the direction of the homolateral nystagmus nor that of the contralateral nystagmus of destruction type was changed in the process and therefore it was considered that the ultrasound may have a directly stimulating effect on the neuro-epithelium and the nerve cells.

METHODS

The ultrasonic apparatus (1.25 MHz) constructed in Uppsala has undergone certain modifications since it was first described (Sjöberg *et al.* 1963). Amongst other things, the original metal tips have since been replaced by tips made of teflon. The method of treatment has also been changed. In the first cases operated on the transducer was directed towards the blue line on the lateral semicircular canal but was later applied to the enchondral bone in the angle between the horizontal and the superior vertical semicircular canals. The operation cavity is kept dry during the operation but a drop of fluid is placed on the tip of the transducer in order to facilitate the ultrasonic transmission. The clinical results have been published by Sjöberg & Stahle (1965).

Experimental studies of the temperature in human temporal bones during ultrasonic irradiation have been carried out since 1963, and the effect of changes in the apparatus and irradiation technique has been specially analysed. The temperature was measured by thermo-couples made of copper and constantan (0.2 mm) which were applied to different parts of the inner ear *and to the facial nerve. It was recorded by* an automatic temperature recorder of the potentiometer type (Speedomax, Leeds & Northrup).

The temporal bone specimens were preserved by deep-freezing until they were to be used when a radical mastoidectomy was carried out including the removal of the auditory *ossicles and the preparation of the labyrinth.* Before any thermo-couple was introduced through the oval window a check was made that the perilymph was still present. In order to obtain a temperature in the temporal bone specimens roughly equal to the body temperature, the specimens were either immersed in the surface layer of a water bath with *a constant temperature of 40 C or kept* in an air filled receptacle coupled to a warm air fan and maintained at 40 C by thermostat control. By both these methods a constant temperature was obtained at each individual measuring point on the temporal bone specimen, but it proved to be impossible to obtain exactly 37 C at all the measuring points at the same time. *The range of values was maximally 3 C.*

In three cases temperature measurements were made in the temporal bone *in vivo* in connection with ultrasonic irradiation. Thermo-couples

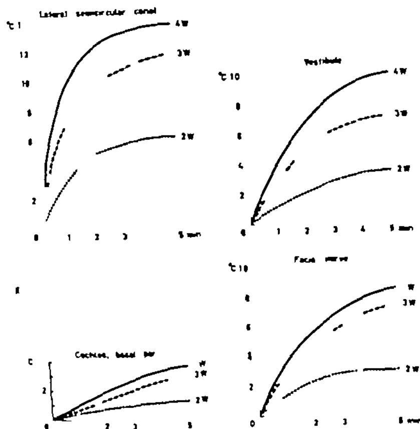


Fig. 3. A similar experiment to that in Fig. 2 but using transducer with teflon tip diameter 2.5 mm and temporal bone immersed in the surface layer of thermostatically controlled water-bath.

3 and 4 watts respecti ely On the basis of an assumed critical temperature of 46 C in the facial nerve the risk of provoking facial palsy would arise fter 4 minutes continuous irradiation with a power of 4 watts, while the critical t mperature would not be attained during 5 minutes continuous i radiation with powers of 2 or 3 watts.

The ultrasonic transducer with the teflon tip (diameter 2.5 mm) applied t the enchondral bone in the angle between the lateral and the superior ertical semicircula canal produced roughly the same rise in temperature as the transducer with the metal tip (Fig. 3) In the vestibul a some what greater rise in temperature was obtained with the teflon tip than w th the metal tip with a power of 4 watts, while the st te of affairs in the cochlea was the opposit with thi power the temperature rise was greater with the metal tip than with the teflon. However the differences were too small for any decisi importance to be asigned to them. The rise in temperature of the facial nerv w s not of such an order of magnitude

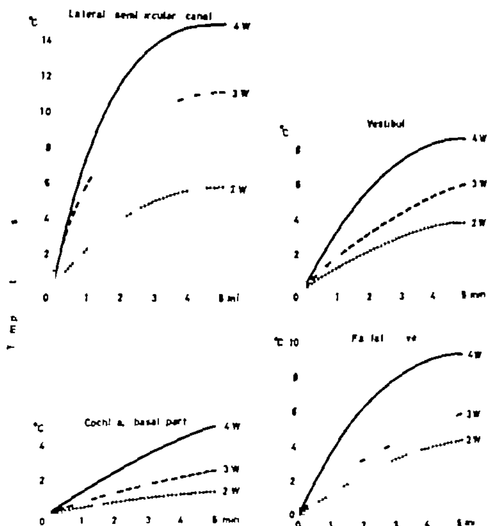


FIG. 2. The rise in t mperature in the lateral semicircul canal, the vestibule the basal part of the cochl a a d the fa ial nerv of a t mporal-bo e peclmen duri g ultrasonic irradi ti n with powers f 2, 3 and 4 watts re pecti ely directed toward the enchondral bone in the angl between the l teral and the superi vert cal semicircular canals. The specimen wa kept in a warm-air recepta le The metal tip had diameter of 2.5 mm.

the superior vertical semicircular canals. The ultrasonic power was varied between 2 and 4 watts The rise in temperature above the initial value measured at each measuring point is illustrated in Fig 2 In the lateral semicircular canal with the thermo-couple introduced from the posterior circumference temperature rises of 6 11 and 15 C, with outputs of 2, 3 and 4 watts respectively were measured after 5 minutes irradiation In the vestibule with the thermo-couple introduced through the oval window the corresponding values were 4 6 and 8.5 C In the basal part of the cochlea, with the element introduced through the round window the temperature rise was small amounting to 5 C after 5 minutes irradiation with a power of 4 watts. In the section of the facial nerve that lies behind the oval window the temperature rise was of the same order of magnitude as that in the vestibule and amounted to 4 6 and 9 C with powers of 2

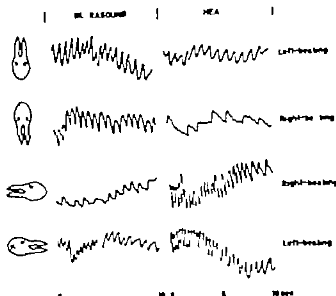
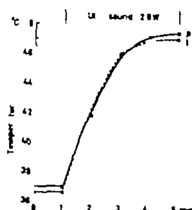


Fig 4 Nystagmogram from rabbit xamined i different positions, partly i connection with ultrasonic irradiation with power of 2.5 watt from metal tip nd partly i connection with irradiation with heat supplied by the same transducer containing w ter at 70°C) towards the left lateral semicircular canal I each position the direction of the nyst gmus was the same, whether ultrasound or heat wa supplied. A chang of position of 180° led t the ystagmu beating i the oppos te direction t the original, whether the hange wa from the normal position t the recumbent position or from the right side t the left ide.

transducer (teflon tip 2.5 mm) was directed towards the enchondral bone in the angle between the lateral and the uperior vertical semicircular canals. The temperature rise was considerably more pronounced in the lateral semicircul r canal than in the vestibule with roughly the same power fr m the transducer (Fig. 5) In the lateral semicircular canal a temperature f 40 C was reached after 3 minutes treatment at 4 watts. In comparison with the measurements made on the specimens irradiated by the same ultrasonic transducer at the same power roughly the same rise in t mperature was btained *in vivo* as in specimen at lower powers, while the temperature rise with higher powers was m re pronounced in the pecimen than *in vivo*

Th relation between the thermal ffect of ultrasound and nystagmus

In order to study whether the homolateral nystagmus which ppears initially during ultrasonic treatment is a caloric reaction induced by the heat or b n ther rigin, experiments were made that were similar in principle to those published by Arslan *et al* (1963) An atticotomy was perf rmed on a rabbit and the middle ear was opened. The rabbit was then placed in a rev lving wooden box mounted on a tand. Its head was



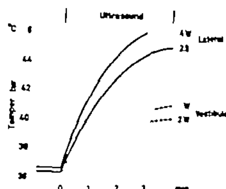


Fig. 4

Fig. 5

Fig. 4 The temperature in a small cell in the bony wall of the l teral semicircular canal (*l. s. c.*) and of the posterior ertical semicircular canal (*p c.*) during ultrasonic irradiation with a pow of 2.8 watt in th angle between the lateral and the uperior vertical semicircul r canal in a patient with Menière disease The metal tip had diamet r of 2.5 mm

Fig. 5 The t mperature in the lateral semicircula canal and in the vestibule during ultrasonic irradiati n at different powers towards the angle between the lateral and the superio v rtical semicircul c nals in a patient with Menière disease The measurem nts w re made in connecti n with operati e destructi n f the labyrinth. Irradiation with tefl n tip (diamet r 2.5 mm)

that an assumed critical temperature of 46 C, i e an increase of 9 C would be reached during 5 minutes treatment with any of the powers used With a power of 4 watts the rise in temperature was greater in the vestibule than in the facial nerve while there were no differences between the rises at both these measuring points with powers of 2 or 3 watts

Temperature studies on patients undergoing irradiation

More conclusive information on heat dispersion can probably be obtained by making measurements on the patient during the irradiation As far as is known, no such studies have been previously reported in the literature

Fig 4 shows the temperature in two bone cells in the walls of the operation cavity one on the posterior vertical semicircular canal and the other on the lateral semicircular canal during ultrasonic treatment in the angle between the lateral and the superior semicircular canals At both measuring points the rise in temperature was almost identical and a temperature of 4⁄ C was recorded after irradiation at 2.8 watts for 5 minutes. A similar experiment on another patient yielded roughly the same result

Of greater interest are the measurements which were made on a patient in connection with a labyrinthectomy on account of Menière s disease After mastoidectomy and preparation of the semicircular canals, together with tympanotomy and stapedectomy a thermo-couple (0 2 mm copper constantan) was inserted into the vestibule through the oval window and was later moved to the opened lateral semicircular canal The ultrasonic

water at 70 C to circulate through it while the ultrasonic generator was switched off When the transducer was then directed to the lateral semicircular canal, with the rabbit in different positions, nystagmus occurred in each position with the same direction as when ultrasound was supplied through the transducer The experiments were repeated on several rabbits with the same result.

In 4 patients with Menière s disease who were undergoing ultrasonic treatment, the direction of the nystagmus was studied partly with the patient lying on his back with his face upwards (supine) and partly with the patient lying on his stomach with his face downwards (prone) (Fig 7) In the former position the ultrasonic irradiation was accompanied by a homolateral nystagmus, while ultrasonic treatment with the patient prone produced a contralateral nystagmus. On the other hand, the contralateral nystagmus of destructive type which appeared later after about 10 minutes treatment did not change direction when the patient turned over but beat towards the healthy ear both in the supine and the prone positions.

DISCUSSION

The difference in ultrasonic power used in our work, as compared with the works reported in Table 1 is to a large extent a result of a difference in technique We do not, as a rule apply our probe tip to a blue line or a comparatively thin layer of bone and therefore use a higher energy level On the other hand, the basic construction of our tip together with the total elimination of extraneous liquid in the operating cavity permits the energy which, because of the impedance differences between the coupling liquid and the bone does not enter the inner ear to return to the tip and be absorbed in the cooling water

Schlieren photographs have shown that the energy distribution across the tip membrane is very unif rm, so that we can justifiably make the assumpti n that the actual point-to-point intensity at the tip is given pproximately by the quotient power/tip area For the range of powers we use (2–4 watt) the intensity runs from 40 to 80 watts/cm Measurements by Kossoff Khan & Farrant (1964) for instance, show that the intensity in the centre of the pen tip used in their instrument is of the same order f m gnitude but is concentrated in a much smaller area That this is also true f the Angell James apparatus c n most certainly be expected.

By virtue of our uniform high-intensity beam we are less dependent upon accurat placement on a very thinly worked section of the canal wall (blue lin) and re able t send the beam through a more massi e ench ndral bone a saf distance from the facial nerve and with less risk of causing a fi ure in the remaining layer of the otic capsule

The results f the temperature measurements in the temporal bone specimen in connection with ultrasonic irradiation cannot be indiscri minately transferred to the conditions during the ultrasonic treatment of

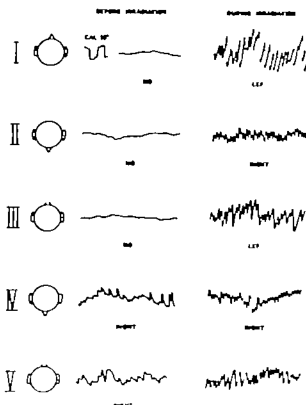


Fig. 7. Nystagmogram from a patient with Menière's disease during ultrasonic irradiation of the left ear with a power of 8 watts from a teflon tip. The recordings were made in both the supine and the prone positions. During irradiation in the supine position (I) with the face upwards a left beating nystagmus appeared. Irradiation in the prone position (II) with the face downward led to a right beating nystagmus, while renewed irradiation in the face-up position (III) produced left beating nystagmus once again. After about 10 minutes ultrasonic treatment, a right beating nystagmus of destruction type appeared and this nystagmus had the same direction in the prone position (IV) and in the supine position (V). During ultrasonic irradiation in the prone position (IV) this right beating nystagmus was strengthened, while irradiation in the supine position (V) produced a change to left beating nystagmus.

outside the box but was fixed to it by a frame which eliminated cervical reflexes. The ultrasonic transducer was directed towards the anterior part of the lateral semicircular canal. Electronystagmography was performed with needle electrodes introduced subcutaneously. The examinations took place with the rabbit placed in different positions in relation to the horizontal plane.

Fig. 6 shows the results after ultrasonic treatment of the left ear. When the animal was turned through 180° a nystagmus was obtained which beat in the opposite direction from the original. In the same experiment the same transducer was also used purely as a heat transducer by allowing

water at 70 C to circulate through it while the ultrasonic generator was switched off When the transducer was then directed to the lateral semi circular canal, with the rabbit in different positions, nystagmus occurred in each position with the same direction a when ultrasound was supplied through the transducer The experiments were repeated on several rabbits with the same result.

In 4 patients with Menière s disease who were undergoing ultrasonic treatment, the direction of the nystagmus was studied partly with the patient lying on his back with his face upwards (supine) and partly with the patient lying on his stomach with his face downwards (prone) (Fig) In the former position the ultrasonic irradiation was accompanied by a homolateral nystagmus, while ultrasonic treatment with the patient prone produced a contralateral nystagmus. On the other hand, the contralateral nystagmus of destructive type which appeared later after about 10 minutes treatment did not change direction when the patient turned over but beat towards the healthy ear both in the supine and the prone positions.

DISCUSSION

The difference in ultrasonic power used in our work, as compared with the work reported in Table 1 is to a large extent a result of a difference in technique We do not, a a rule, apply our probe tip to a blue line or a comparati ely thin layer of bone, and therefore use a higher energy level On the other hand, the basic construction of our tip together with the total elimination of extraneous liquid in the operating cavity permits the energy which, because of the impedance differences between the coupling liquid and the bone, does not enter the inner ear to return to the tip and be absorbed in the cooling water

Schlieren photographs have shown that the energy distribution across the tip membrane i very uniform, so that we can justifiably make the assumption that the actu l point to-point intensity at the tip is given approximately by the quotient power/tip area. Fo the range of powers we use (2–4 watts) the intensity runs from 40 to 80 watt cm Measurements by Kossoff Khan & Farrant (1964) for instance show that the intensity in the centre of the open tip used in thei instrument is of the same order of magnitude but is concentrated in a much smaller area That thi is also tru f the Angell James pparatus can most certainly be expected.

By irtue of our uniform high-intensity beam we are less dependent upon accurate placement on a very thinly worked section of the canal wall (blue line) and are able to send the beam through a more massive enchondral bone a saf di tance from the facial nerve and with less risk of causing a fissure in the remaining layer f the oti capsule

The results of the temperature measurements in the temporal-bone specimens in connection with ultrasonic irradiation cannot be indiscriminately transferred to the condition during the ultrasonic treatment of

NYSTAGMUS IN FACE-UP AND FACE DOWN POSITION
BEFORE AND DURING ULTRASONIC IRRADIATION OF
THE LEFT EAR

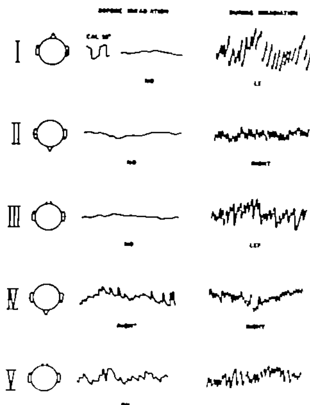


Fig 7 Nystagmogram from a patient with Menière s disease during ultraso ic irradiati n of th l ft e with a powe f 3 watts from a t fl n tip The recording were made in both the upine d the pron positions. Du i g irradi ti in the supine po iti n (I) with the f ce upw rd left be ting nyst gmu ppe red Irradi ti in th prone po iti n (II) with th f ce d wnwards led to right beating ny tagmus, whil renewed irradi ti n in the f ce-up position (III) produced left beati g ny tagmus one g in After bout 10 min t ultraso i treatment, a ight-beati g nystagm of d tructi type appeared d thi nystagm had the sam directi n in the prone po ition (IV) a in the upine po iti n (V) Duri g ultraso ic irradi ti n in the prone po ition (IV) thi right beati g nyst gm w s strengthened, whil irradi ti n in the supi positi n (V) produ ed change to l ft-beating t gmus.

outside the box but was fixed to it by a frame which eliminated cervical reflexes The ultrasonic transducer was directed towards the anterior part of the lateral semicircular canal Electronystagmography was performed with needle electrodes introduced subcutaneously The examinations took place with the rabbit placed in different positions in relation to the hori zontal plane

Fig 6 shows the results after ultrasonic treatment of the left ear When the animal was turned through 180 a nystagmus was obtained which beat in the opposite direction from the original In the same experiment the same transducer was also used purely as a heat transducer by allowing

are therefore a probable explanation of the nystagmus induced during ultrasonic irradiation, as distinguished from the contralaterally directed nystagmus of destruction type which does not change direction when the patient is prone

RÉSUMÉ

Les températures rele ées sur un os temporal humai isolé soumis à l irradiation ultra-sonore montrent que la hausse de température est a ssi forte sinon plus fort dans le vestibule que dan le nerf facial, quand l'émetteur est appliqué sur l'os enchondral d n l ngle formé pa le canal vertical d mi-circulaire de devant et le canal latéral tandis que les études citées dan la littérature avec l'émetteur appliqué sur la ligne bleue du canal latéral, ont souvent montré des temperatures plus élevées dan le nerf facial que dans le estibule La hausse d température était faible dans le limaçon Les mesures de température dans le vestibule et le canal latéral t montré que pendant l'irradiation ultra-sonore d'un malade att int du sy drome de Menière le résultat pour des ffet ultra-sonore plus faibles était à peu près le même que celui obtenu dans les études sur des os temporaux isolés, alors que pour des ffets plus élevés la hau se de température était moins importa te i ivo que dans la préparation.

Le traitement à l'ultra-son des lapins et des hommes, placés dans différentes positions, montre que le ystagmus qui se produit pe d t l'irradiation est vraisemblablement une réaction calorifique provoquee par l ffet thermique de l'ultra-son

ZUSAMMENFASSUNG

Temperaturmessungen an isolierten menschlichen Schläf nbeinen bei Ultraschallbestrahlung ergaben dass bei Anbringu g des Strahlers am encho dralen Knochen im Wink l zw schen dem vorderen vertikalen nd dem lateralen Bogengang die Temperaturst igerung im Vestibulum ebenso gross wie oder grösser h im Nervus fa ialis war wahrend die in der bisherigen Literatur vorgelegten Studien, w der Strahler n der blauen Linie des lateralen Bogenga gs appliziert war durchgehend höhere Temperaturen im Nervus f cialis als im Vestibulum erhalten hatten In der Cochlea stieg die Temperatur nur wenig an. Temperaturmessungen m Vestibulum und im lateralen Bogenga g bei Ultraschallbestrahlung ines Patienten mit Menièrescher Krankheit ergaben bei geringer Energie des Ultraschalls ungefahr dieselben Resultat wie die U tersuchungen an isolierten Schläfenbeinen, während bei stä keren Energien die Temperaturerhöhung in vivo geringer war als an den Präparaten

Die Ultraschallbehandlung vo Kaninchen und Menschen i verschied en Lagen ze gte, dass der bei der Bestrahlung a ftretende Nystagmus wahrscheinlich ine vom thermischen Eff kt des Ultraschalls hervorgerufen kalorisch Reaktion ist.

patients Post mortem changes, including the absence of blood circulation, the removal of the surrounding tissues and the opening of the inner ear mean that the conditions are not equivalent to those during operations and in the ultrasonic treatment of patients with Menière s disease The difficulty of obtaining an initial temperature in the specimen corresponding to that *in vivo* has a detrimental effect upon the results of studies of specimens. It is true that these problems are eliminated in temperature measurements on experimental animals, but the value of such experiments is reduced by the fact that the anatomy of the animals is not the same as that of man

The temperature studies on temporal bones of human beings (*in vivo*) and those on temporal bone specimens showed great similarities at low ultrasonic powers, while at higher powers there was a smaller rise in temperature *in vivo* than in the specimens, possibly due to the fact that the dissipation of heat energy through the circulating blood was relatively pronounced at higher powers.

The relatively insignificant rise in temperature in the cochlea is in agreement with the clinical results, that after ultrasonic treatment of patients with Menière s disease the hearing in the majority of cases is unchanged

When the ultrasonic transducer is directed at the angle between the lateral and the superior vertical semicircular canals, instead of at the lateral semicircular canal the distance from the facial nerve is increased. The risk of thermal damage to the nerve is thereby decreased, while the distance to the vestibule is reduced In the studies reported in the literature about the temperature in the temporal bone during ultrasonic treatment, the rise in temperature was considerably greater in the facial nerve than in the vestibule (Table 1) while the method and apparatus used in the present investigation produce an equally large increase in temperature at both these points and an even greater increase in the vestibule than in the facial nerve, when high powers are supplied through the teflon tip

In order to reduce the risks of facial palsy during the ultrasonic treatment of patients with Menière s disease, pauses are always made in the treatment after 2–3 minutes of continuous irradiation especially when high powers are being used By this means the temperature in the facial nerve is prevented from reaching the critical value

The experiments on rabbits showed that the homolateral nystagmus induced by ultrasound follows the same pattern as the caloric reaction induced by heat Certain differences in relation to the nystagmus directions in different positions reported by Arslan *et al* (1063) can probably be explained by the fact that in our experiments the transducer was applied nearer the ampulla on the lateral semicircular canal The present studies on human beings show that during ultrasonic irradiation with the patient supine the nystagmus beats towards the operated ear while irradiation with the patient prone produces nystagmus which beats from the treated ear Endolymph currents caused by the thermal effects of the ultrasound

Acta oto-laryngologica 64 477–491 (1967)

ADENOSINE TRIPHOSPHATASE DISTRIBUTION IN THE ORGAN OF CORTI

Histochemical Study by Light and Electron Microscopy

Y Nakai and D Hilding
New Haven Conn U.S.A

From the Otolaryngology Section and Otologic Research Laborat ry Yale University School f Medi ine New H ven

Adenosi e triphosph tase (ATP-ase) pro ides nergy f r active tran port acros cell membranes by its hydroly i of Adenosi etriphosphate t Adenosinediphosphat ATP-ase generally is found where acti trans-port of water nd electrolytes occurs. We used i listochemical m thod to study the location of this enzyme withi the organ of Corti. W found that the outer surface of most cells in Corti' organ show enzyme ac-tivity No ATP-ase was found alo g th side of hair cell where they are in contact with cortilymph or between adjacent nerve endings. Supporting cells ha the en yme on their entire surface The enzyme was found i the basement membrane of th spiral vessel. T n l-crossing nerve fibers hibited enzyme cti ity on their outer mem bra e The enzyme wa located on the end lymphatic layer of R issner's membrane cells, but not on the perilymph tic layer

INTRODUCTION

Adenosine triphosphatase (ATP-ase) can generally be found where sodium and potassium are being actively transported across cell mem branes. Therefore locati g the sites within the organ of Corti where this enzyme occurs sh uld clarify the way that hair cell maintain their fluid and electrolytes. In turn this inform tion could aid in understanding inner ear d sorders.

Th hair cells of the organ of Corti presumably obtain their electrolytes from urrounding cell and fluids. Th hairs and upper surface of the hair cell are in contact with endolymph while most of the rest of the cells' surfa e is bathed in "Cortilymph" The lower part of the cell rests in a cup formed by supporting cells and nerve endings. Thus, several alternative supply routes a e possibl

This tudy was supported by Public Health Service Research Grants from the N tional I stitut of Neurological Diseases nd Blindness (NB 03985 and NB 06133-01) nd grant from the Deafness Research Foundation

REFERENCES

ANDERSON T O WAKIM, K. G HERRICK, J F BENNETT W A and KRUSEN, F H, 1951 An experimental study of the effect of ultrasonic energy on the lower part of the spinal cord and periph ral cord nerves. *Arch. Phys Med* 32 71

ANGELL JAMES, J 1963: New dev lopments in the ultrasonic therapy of M nière's disease *Ann Roy Coll Surg Engl* 33 226

ANGELL J MES, J DALTON, G A FREUNDLICH, H F BULLEN, M A WELLS, P N T HUGHES, D A and CHOW J 1964 Histological, thermal and biochemical effects of ultrasound on the labyrinth nd temporal bone *Acta Otolaryng* (Stockh.) 57 366

ANGELL JAMES, J DALTO G A HADLEY K J FREUNDLICH, H F BULLEN, M. A and WELLS, P N T 1963: A new 3-megacycle generator fo destruction f the vestibular end organ. *Acta Otolaryng* (Stockh.) 56 148

ARSLAN, M SALA, O and MOLIN RI, G 1963 The ultrasonic irradiatio of the posterior labyrinth in Menière disease *Acta Otolaryng* (Stockh.) 56 154

BASEK, M and EPANCHIN V N 1964 New ultra nic generator for treating Menière's disease *Arch Ot laryng* (Chic.) 80 271

BULLEN, M A WELLS, P N T FREUNDLICH H F and A GELL JAMES, J 1963: A physical rvey of the ultrasonic treatment of Menière's disease *Ult sonics,* 1 1.

KOSSOFF G KHAN A F and FARRANT R H 1964 The use of the C. A. L. ultrasonic gen rato in M nière di ease *J Laryng* 78 245

SJÖBERG, A nd STAHLE, J 1965 Treatment of Menière's disease with ultrasound. *Arch Otolaryng* (Chi) 82 498

SJÖBERG, A STAHLE, J JOHNSON S nd SAHL, R 1963: Treatment of Menière dise se by ultrasoni irradiati n Phy ical, experim tal and linical studies. *Acta Otolaryng* (Stockh) S ppl *178*

STAHLE, J and SAHL, R 1964 Electrony tagmography i Me ière's disea e bef re du ing nd fter ultra o i irradiati n. *Acta Otol ryng* (Stockh.) S ppl *192* 1 4

Dept of Otolaryngology University H pit l
Uppsala 14 Sweden

Received May 2 1967

(2) Tissues were fixed for 30 minutes in cold 2 per cent glutaraldehyde buffered to pH 7.2 with 0.05 *M* cacodylate After this prefixation the same procedures were followed with incubation in Wachstein Meisel reagent (above) for 15 minutes.

After incubation all tissue samples were washed in cold 0 44 mol sucrose and then fixed additionally in 1 per cent osmium tetroxide, buffered to pH 7.2 for 1.5 hours, dehydrated in alcohol, and embedded in Epon plastic. Thin sections were cut with an LKB ultratome mounted on formvar coated copper grids, and examined without any additional staining in an RCA EMU 3G electron microscope

The same procedures were followed in other experiments in which ATP in the incubating medium was replaced by β-glycerophosphate adenosine monophosphate (AMP) adenosine diphosphate (ADP) or inosine triphosphate (ITP)

The replacement of tris-maleate buffer by tris-HCl buffer with ouabain 1mM added was done to tudy the inhibiting effect of reduced sodium and potassium ions in the presence of ouabain. We also studied the activating effect of incubation with increase of potassium chloride to 30mM

Control specimens were prepared by following each of the procedures as outlined above with the exception that ATP was omitted from the incubation medium.

Pieces of the organ of Corti were examined with the light microscope. The tissues were removed from the incubating medium, washed in buffer and treated with 1% ammonium sulfide to develop a visible black precipi tat (lead sulfide) from the reaction product, (lead phosphate) and examined without embedding and sectioning.

Because the accumulate precipitate of lead phosphate is opaque in the electron beam, conversion to lead sulfide was omitted for electron microscopical study

RESULTS

When tissues were incubated with ATP as substrate, activity of the enzyme was obvious at first glance by light microscopy Practically all the structures of the rgan f Corti in every turn showed a brownish stain. The high power f the light microscope revealed presence of reaction mainly in the region of the intercellular borders of the hair cells and supporting cells and hairs of the hai cells, but its resolution was inade quat to determine the exact site f deposition.

In lectron microscopic observations, it was with formaldehyde fixation that the most general localization of reaction deposits was observed, but to obtain intact fine structure glutaraldehyde pre-fixation is required Unfortunately glutaraldehyde also interferes with enzyme activity Fig. 1 summarizes our findings diagramatically

When tissue was fixed in glutaraldehyde for 30 minutes and then in-

The histochemical study of ATP-ase is complicated because biochemical studies have demonstrated that at least three different enzymes split ATP Mitochondrial ATP ase is important in the metabolism of carbohydrate A critical concentration of calcium is required by the second kind of ATP ase which is found in muscle and contributes energy for muscle contraction Fluid transport across cell membranes is the role of the third type of ATP ase which is dependent on a critical concentration of sodium and potassium (Pearce, 1961 Burstone, 1962 Schwarz, Bachelard & McIlwain, 1962 Skou 1962) These facts must be taken into consideration in the interpretation of histochemical findings. The presence of ADP ase and other nucleoside phosphatases is another problem they often produce a reaction deposit with the reagents we used After the reagent ATP has been split to form adenosine diphosphate ADP has substrate, and its activity will be added to that of the ATP ase in the formation of reaction deposit Other authors (Torack & Barrnett, 1963 Marchesi Sears & Barrnett, 1964) have used the term nucleoside phosphatase" instead of ATP ase to avoid misleading the reader about the specificity of this method. We believe that most of the enzyme activity revealed by this technique in the organ of Corti is due to ATP-ase and prefer to designate it ATP ase for the sake of clarity

The type of pre fixation used and the length of incubation in the reagent mixture influences the way reaction deposit appears. These factors were discussed in our earlier paper on the localization of ATP-ase in the stria vascularis (Nakai & Hilding 1966) As a result of these observations, we used formaldehyde or glutaraldehyde as pre fixatives in the present study

The present report is concerned with the histochemical localization of ATP ase activity of the organ of Corti

MATERIALS AND METHODS

The inner ear structures of anaesthetized guinea pigs were removed and immediately placed in fixative solution Much of the bone surrounding the cochlea was dissected away and Reissner s membrane was torn to expose the organ of Corti to the chemicals

In the preparation of tissue the following procedures were employed

(1) The specimens were fixed in cold 10 per cent formaldehyde with 4 9 per cent sucrose buffered to pH 7.2 for one hour Following this prefixation the tissue was washed in several changes of cold 0 1 mol cacodylate buffer (pH 7.2) containing 0 44 mol sucrose for 3 hours Then the specimens were put into the reagents for histochemistry The incubating medium employed was the Wachstein & Meisel solution (1957) 8.3×10^{-4} M ATP (disodium salt) 0 04 M Tris maleate buffer (pH 7 2) $3\,6 \times 10^{-3}$ M Pb $(NO_3)_2$ and 0 01 M $MgSO_4$ were added together in the above order and constantly stirred for 15 minutes at room temperature then filtered The tonicity of the filtrate was adjusted by adding 0 4 mol sucrose


HC
2
HC
3

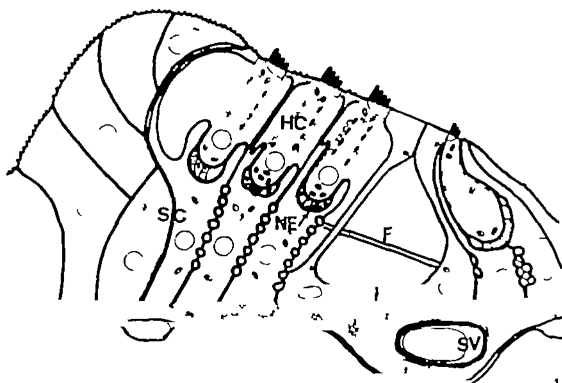


Fig 1 Th heavy line in thi di gram wa drawn wherever we f und ATP-ase cti ity. The enzym is located on the s rface of supporti g cell (*SC*) nd nerve fibers (*F*) Hair cells (*HC*) h v th enzyme nly on th ir upper end cept where the touch supporting cells and nerve endings. Enzymatic cti ity wa f u d in the basement membrane surroundi g th piral vessel (*SV*) Nerve ending (*NE*)

cubated in the reaction mixture with ATP as substrate prior to further osmium fixation the reaction precipitate was concentrated on the outer surface of the hair and upper surface of the inner and outer hair cells (Fig 2) In addition to the activity on the hair surfaces, activity appeared between membranes of the hair cells and adjacent supporting cells Enzyme activity was absent on the plasma membrane of the hair cells where they touch Cortilymph

Dense reaction product filled the space between the plasma membrane of the hair cells, supporting cells, and nerve endings However where only nerve endings were opposed little reaction product was deposited in the interspace (Fig 4) The outer surfaces of the tunnel fibers exhibited enzyme activity (Fig 5)

Fig. 2. Electron micrograph showi g reacti product th rf ce f th ha rs nd uppe surface of an o ter h i ll (*HC*) Gl tarald hyd pre fi ti n and i cubatio with ATP a substrat f r 15 mi tes.

Fig 3. The urface f each h i f th in er hai cell (*IHC*) i reacti e N tice th thick layer of reacti n deposit at th surf ce f th cuticul pl t (*arrow*) Very dense d posit was f und o th supporti g ll f rmi g the i ne border of these hair cells. F rmaldehyd pre-fixatio and ncubati with ATP f 15 mi utes.



Fig. 8. Pillar cell mitochondri demonstrating ATP-ase within mitochondria w th formaldehyde pre fixation (*arrows*) Incubation with ATP for 15 mi utes

Fig. 9 The spiral essel beneath the basilar membrane has marked activity i the region of its basement membrane (*arrows*) Surrounding cells nd tissu lso show activity Formaldehyde pre-fixation and incubation w th ATP for 15 min tes.

Fig. 10. Reissner' membrane The side facing endolymph ha ATP ase (*arrows*) That in contact with perilymph show no reaction Scal media (*SM*) Scal estibuli (*ST*) Glutaraldehyde pre-fixatio nd incubation with ATP

The supporting cell's plasma membr ne was quite reactive Microvilli cover the outer surface of Hensen s cells increasing their surface area Marked enzymatic activity (Fig 7) of these microvilli was found. This tends t support Engström & Wersäll s (1953) notion th t they are part of the route for supplying hair cells.

When prefixation was done with formaldehyde-sucrose (Fig. 3) reac tion product was found in the same places in the organ of Corti as with glutaraldehyde (Fig. 6) In addition to these sites, reaction product could

Fig. 6. The outer surface of each Deiter cell (*DC*) ha ATP-ase, but hair cell (*HC*) d not except t their top and where they touch Deiter cells (*arrow*) Both efferent nd afferent nerv fibers are coated with reaction product. Glutaraldehyde pre-fixation nd ATP incubation for 15 minutes.

Fig. 7 Each microvillus of Hensen cells i coated with reaction product Notice the bilaminar character of the deposit on the outer cell surface (*arrow*) Glutaraldehyde pre-fixation nd ATP incubation for 15 minutes.



FIG. 4 Lower porti f n outer h l cell (*HC*) h wi g how the reacti n d po it is distribut d o the o t ll urf ce f th hair ll d ighbo ring upporti g cell (*SC*) Each nerve fibers (*F*) i coated with reactiv materi l and there i deposit i the space between upporti g d nerv di gs. (*NE*) Gl t rald h d pre-fixat nd 15 minutes incubation with ATP

FIG 5 Tunn l-crossi g fibers h reacti ter rf ce Pres mably ATP se used fo returni g pota ium t with th rv fibe g i t conce tration gradi t The presence of ATP-ase th rf ce f these fibers (*ar ws*) i directl suggests that th y are surrounded by fluid f rel ti ly l w pot i m nce trat n Glut ralde-hyde pre-fix tion nd 15 minutes incubati n with ATP



Fig. 11. Control specimen incubated without substrate. Upper portion of outer hair cell (HC). No enzyme activity.

Fig. 12. Control specimen incubated in the medium without substrate after pre-fixation in glutaraldehyde. Hensen cell with no evidence of activity. Centriole (C).

Fig. 13. Part of cell ... of outer hair cell (HC). Note multiple lamellae (L) of outer cell membrane flanked by mitochondria (M). Osmic acid fixation. Uranyl acetate stain.

Formaldehyde and glutaraldehyde differ in their effects on the ultrastructural demonstration of ATP-ase in the organ of Corti. The most general localizing of reaction deposits was observed with formaldehyde fixation. This fixative can be recommended for studying the over-all distribution of this enzyme. As others have reported, we found that glutaraldehyde preserves structures better but interferes with enzymatic activity (Tormey & Barrnett, 1963; Barrnett, 1964; Marchesi, Sears & Barrnett, 1964; Sabatini, Miller & Barrnett, 1964; Nakai & Hilding, 1966).

be demonstrated in mitochondria of hair cells and supporting cells (Fig 8) as well as in Golgi apparatus in hair cells with formaldehyde pre-fixation

The densest reaction product appeared in the region of the basement membrane of the spiral vessel beneath the tunnel of Corti (Fig 9) This acitivity was inhibited by glutaraldehyde prefixation as is basement membrane ATP-ase in cerebrum (Torack 1965) and is only demonstrable with formaldehyde prefixation

Reissner's membrane exhibited enzyme activity on its endolymphatic surface only As illustrated in Fig 10 Reissner's membrane consists of two layers of cells separated by basement membrane There are microvilli on the endolymphatic ectodermal cells, but none on the mesodermal cells that face perilymph Reaction deposit was found on the microvilli and plasma membrane of the endolymphatic side

When sodium and potassium ions were removed from the medium and ouabain added a marked reduction in staining was noted Potassium chloride appeared to enhance ATP ase activity However our cytochemical technique is only a qualitative estimate of enzyme presence and differences in quantity of the enzyme cannot be precisely measured

When ADP (adenosine diphosphate) was substituted for ATP less reaction product was found on the hairs and on the upper surface of the cuticular plate of the hair cells but the distribution was otherwise the same some reaction product on each aspect of supporting cells and on nerve fibers

ITP (inosine triphosphate) as substrate produced a precipitate on supporting cell surfaces, particularly the upper ones. No reaction occurred on the hairs or cuticular plate of hair cells

When β-glycerophosphate or adenosine monophosphate were used as substrates no reliable reaction was observed Sparse deposits were found in some areas, but these bore no constant relationship to cellular components.

In control specimens, incubated without ATP no reaction deposit occurred (Figs 11 and 12)

DISCUSSION

We undertook the present study to reach an understanding of the way in which ATP ase is distributed in the organ of Corti using histochemical techniques by light and electron microscopy

We used whole mounts of the organ of Corti for light microscopy As Engstrom Ades & Hawkins (1962) indicated, it is possible to study large portions of the organ of Corti in this way without embedding or sectioning When we incubated tissues with ATP as substrate the reaction product was found between hair cells and supporting cells and on hairs of the hair cells, but resolution was inadequate to determine the exact site of deposition

physiological position (Hilding, 1952) endolymph cannot freely reach the tops of the hair cells. Therefore the rather intense activity of ATP-ase in this relatively confined sub-tectorial membrane space is difficult to understand. Equally intriguing is the absence of ATP ase activity on the cell wall in contact with Cortilymph The line-up of mitochondria and the lamina of smooth membranes along the inner cell surface of this region suggests that a high level of metabolic activity characterises this portion of hair cells (Fig 13) In the past, most of us have thought this to be a region of transport for substances from Cortilymph. Instead, our finding that ATP-ase is absent suggests that the inner lining of membranes and mitochondria is a system engaged in moving substances from one end of the hair cell to the other One wonders if this system is concerned with transmission of bits of information, possibly coded chemically from the bent hairs at the top to the nerve endings at the bottom.

At the basal portion of hair cells is the important junction with the terminal nerve endings of the cochlear nerve and of the bundle of Rasmussen. Reaction deposit was located in the synaptic cleft between the plasma membrane of hair cells and nerve endings as well as between these endings and adjacent Deiter cells. There was no enzyme activity between adjacent nerve endings. These findings are similar to those reported by Marchesi, Sears & Barrnett (1964) in retina and brain. They reported absence of nucleoside phosphatase activity in the space between neural elements, but said that it is present between neuronal and glial membranes and between adjacent glial membranes. Barrnett (1962) thought that ATP ase in the spaces around nerve endings suggests that they are a site for production of neurotransmitter

The outer surfaces of the tunnel fibers show ATP-ase activity Presumably these fibers are like neuronal tissue everywhere in releasing a comparatively large amount f energy from high energy phosphate bonds by means f ATP ase in order to restore intercellular potassium after it has been lost during transmission of nerve impulses. Thus, the presence of ATP-ase in the cell membrane of the tunnel crossing fibers furnishes indirect evidence that the fibers are surrounded by low-potassium fluid otherwise energy would not be required because potassium would not be movi g against a concentration gradient. The presence of ATP-ase on the outer surface of the tunnel crossing fibers suggests that the fluid within the tunnel of Corti contains relati ely low potassium like perilymph rather than high potassium of endolymph. This indirectly supports the observations of Rauch (1964) Tasaki Davis & Eldridge (1954) and Tonndorf Duvall & Rene u (1962) and others that the tunnel of Corti is filled with perilymph rather than endolymph.

Chemical tudies on cell fractions have indicated that another type of ATP-ase i generally present in mitochondria (Padykula & Herman, 1955) W found activity with ATP as substrate localized on the cristae of mitochondria in every kind f cell within the organ of Corti when formal

Generally neurons of the central nervous system are separated from their blood vessels by supporting cells. Torack & Barrnett (1963) said that glial cells are interposed between neurons and extracellular fluid to carry nutrition and oxygen to neurons They reported that these intermediary cells were the site of ATP ase activity We have found a similar pattern in the relationship between hair cells, supporting cells and spiral vessel beneath the basilar membrane ATP ase was found on supporting cells which lie between the spiral vessel and the hair cells.

The outer surface of Hensen cells are covered with small microvilli where they are in contact with endolymph Engstrom & Wersäll (1953) said that their presence suggested that this surface of supporting cells was engaged in fluid transport Abundant ATP ase activity on this membrane with its microvilli is evidence of active transferral of substances across the boundary with endolymph

ATP ase activity was reduced by elimination of sodium and potassium from the incubating medium showing that at least part of the ATP ase activity demonstrated on the plasma membrane in the organ of Corti is a sodium, potassium activated ATP ase This is the type of ATP ase which is involved in active transport across membranes generally (Baker 1966)

ATP ase activity was localized in the basement membrane region surrounding the spiral vessel beneath the organ of Corti when formaldehyde-sucrose solution was used for prefixation Marchesi & Barrnett (1964) and Torack (1965) showed nucleoside phosphatase activity in the basement membrane of capillaries in other tissues They said that this enzymatically active basement membrane should be looked upon as a way of facilitating transport rather than as a barrier The spiral vessel beneath the basilar membrane has recently been emphasized as an important source of supply for the organ of Corti by Lawrence (1966) By routes that are not yet clear substances apparently can migrate from the region surrounding the spiral vessel into supporting cells and then into hair cells Kikuchi & Hilding (1966) recently found that the spiral vessel beneath the basilar membrane in Shaker 1 mice underwent early pathological involution ATP-ase activity as tested with this histochemical technique was lacking in the spiral vessel region of Shaker mice tending to confirm its abnormality

Mizukoshi (1966) saw ATP ase activity on the hairs of cochlear hair cells in his recent light microscopy study Vinnikov & Titova (1964) demonstrated by light microscopic histochemical study that the hair cells contains substances with carboxyl groups and alkaline phosphatase They reported that the hairs of the organ of Corti contain carboxylic groups, alkaline phosphatase acid phosphatase phosphorylase and acetylcholin esterase We found that the plasma membrane of the hairs and upper end of the hair cell was reactive

The tops of the hair cells are exposed to fluid space between the tectorial membrane and the sides of the hair cells are in contact with "Corti lymph" One would guess that when the tectorial membrane is in its

ATP-ase activity was absent on that major portion of the hair cells along the sides where they are in contact with "Cortilymph" A cylinder of complex membranes lined by numerous mitochondria is found near the hair cell's surface in this region. Absence of ATP-ase in this part of hair cells plasma membrane suggests that the inner special membrane system with its mitochondria is not engaged in transport of ions in or out of Cortilymph but that another purpose for it will someday be found One could speculate about whether this rather complicated special organization is engaged in movement of ubstances from one end of the hair cell to the other Maybe this kind of chemical transfer is associated in some way with the way that hair cell carry information from minimal deformations of the hairs on top to the nerve endings at the bottom

This histochemical technique seems to be promising for evaluation of abnormal inner ear tissue and we hope to apply it to the study of various pathological states

ZUSAMMENFASSUNG

Die Lokalisation des Enzym Adenosin Triphosphatase (ATP-ase) wurd m t fels ner histochem schen Method und Elektron mikrosk ple untersucht ATP-ase hat m w htige Rolle m Zellmembra tran port Die Vert ilung der ATP-ase in erhalb des Cortischen Organs mü ste laher ine Bez ehung zu der Aufnahme von Substa zen in die Haarzellen h ben. Wir benutzten die (etwas geänderte) Wachstein-M iselsch M thod f r N kleot dphosphatasen. Reak tionsniederschlag, welcher ATP-ase-Aktivität n gt, wurde hauptsä hlich an der Plasmamembran w lch die Zellen des Cort schen Organ umgibt, gefunden Dann beoba hteten wir jedoch ein ge interessante Ausnahme

Kein Enzy akti ität wurde an den Seiten der Haarzellen, welche m t ler Cortischen Lymphe" in Berührung k mmen beobachtet Die mesothelial Zellen der R issnerschen Membran eigten kein Aktivität w hrend di ectoder malen endolym phatischen Zellen sehr rei hlichen Reakt nsl derschlag auf wiesen

Andere Autoren haben Abwesenheit on ATP-ase zw ischen ben chbarten Neuralelementen (mit Ausnahme des synaptischen Spaltes) beobachtet. I ähnlicher W se kon ten wir k ine Akt ität zwischen den Nervenenden im Cortischen Organ beobachten ATP-ase w r jedoch im synaptischen Spalt zwischen Nerve zellen und Haarzellen vorhanden

Die Enzymakt vität war analog der Akt ität im Gehirn vert ilt Wie im Gehirn die Kapillaren i ihrer Basalmembran eine Form von ATP-ase ufweisen, welches besonders empfindlich gegen V rfi erung mit Glutaraldehyde ist so fanden wir da das Spiralgefäss unter d m Cortischen Organ in seiner Basal membran die gleiche Art von ATP-ase-Aktivität zeigt. Zwischen den Gehirnge fässen und Nervenzellen befi den sich Stütz (Glia) zellen welche ATP-ase-Aktivität zeigen Die Nervenzellen selbst haben jedoch k in Enzym an ihrer Oberfläche Wi beobacht ten, dass di Stützzellen m Cortischen Organ ebenf ll Enzymakti ität haben und dass sich diese Zellen in iner Weise zwischen Gefäs

dehyde sucrose was used for pre-fixation. Siekevitz *et al.* (1958) suggested that this enzyme is probably engaged in oxidative phosphorylation. We have recently studied the pattern of distribution of oxidative enzymes within the organ of Corti and as one would expect succinic deydrogenase and diaphorase can also be demonstrated on mitochondrial cristase (Nakai & Hilding 1967).

SUMMARY AND CONCLUSION

The location of Adenosine triphosphatase (ATP ase) in the organ of Corti was studied by means of a histochemical technique and electron microscopy. This enzyme plays an important role in transport across cell membranes. The way it is distributed within Corti's organ should be related to the way that hair cells obtain vital substances. We used a modified version of the Wachstein Meisel histochemical method for nucleoside phosphatases. With appropriate pre-fixation and incubating medium it showed the sites where sodium potassium activated ATP ase exist. Reaction deposit indicating ATP ase activity was found on most of the plasma membrane surrounding organ of Corti cells with some interesting exceptions.

No enzyme activity was demonstrated on the sides of the hair cells where they are in direct contact with "Cortilymph". No reaction deposit was found on the mesothelial cells of Reissner's membrane but endolymphatic ectodermal cells had abundant deposit. Others have observed absence of ATP ase between adjacent neuronal elements in the brain except in the synaptic cleft. Similarly no activity was demonstrable between nerve endings in the organ of Corti. ATP ase was present in the synaptic space between nerve endings and hair cells.

Enzyme activity was distributed analogously to the brain. As brain capillaries have a form of ATP-ase particularly sensitive to prefixation with glutaraldehyde in their basement membrane so does the spiral vessel beneath the organ of Corti. Supporting (glial) cells with ATP ase activity lie between brain blood vessels and neurons. Neurons do not have the enzyme on their outer surface. We found the supporting cells in the organ of Corti have enzymic activity and are interposed between vessel and hair cell in a way which reminds one of the relation between neurons, supporting cells and vessels.

The uncovered nerve fibers which cross the tunnel of Corti were found to show ATP ase activity. The presence of the enzyme on these fibers indirectly suggests that the fluid in the tunnel has a relatively low concentration of potassium since the principal energy need of neurons is for transport of potassium back into the cell against a concentration gradient after it has been lost during depolarization. If the fluid surrounding these fibers had the same potassium content as endolymph there would be no concentration gradient to require active transport and ATP ase

ATP-ase activity was absent on that major portion of the hair cells along the sides where they are in contact with "Cortilymph A cylinder of complex membranes lined by numerous mitochondria is found near the hair cell's surface in this region Absence of ATP-ase in this part of hair cells plasma membrane suggests that the inner special membrane system with its mitochondria is not engaged in transport of ions in or out of Cortilymph but that another purpose for it will someday be found One could speculate about whether this rather complicated special organization is engaged in movement of substances from one end of the hair cell to the other Maybe thi kind of chemical transfer is associated in some way with the way that hair cells carry information from minimal deformati ns of the hairs on top to the nerve ending at the bottom

This histochemical technique seem to be promising for evaluation of abnormal inner ear tissue and we hope to apply it to the study of various pathological states.

ZUSAMMENFASSUNG

Die Lokalisatio des Enzyms Adenosin Triphosph tase (ATP-ase) wurd n t tels einer h stochemischen Method und Elektronenm kroskople unt rsu ht ATP-ase hat eine wichtige Rolle im Zellm mbra transport. Die Vert ilung ATP-ase innerhalb des Cortischen Organ mu te d her n Beziehung zu der Aufnahme on Subst n en in di Haarz llen haben W benutzten di (etwas geanderte) Wachstein M selsche M thode fur N kleot dphosph t sen Reak tionsniederschlag, welcher ATP-ase-Akti itat anzeigt wurd hauptsa l li h an der Plasmam mbran wel he d e Zellen des Cortischen Organ mg bt, gefun len. Dazu beobachteten wi jedoch e ige interessante Ausnahmen

Keine Enzymakti itat wurd an den Seit der H arzell n w lche mit der Cortischen Lymphe" in Beruhrung kommen beoba htet Die mesoth liale Zellen der Reissnerschen Membran ze gten k in Akti itat w hren l die elod r malen endolymphatischen Zellen seh reichli hen Reaktion nied rschlag f lesen.

Andere Autoren haben Abwesenheit on ATP-ase zwischen ben chbarten Neu ralelementen (mit Ausn hme des ynaptischen Spalt) beobachtet I l h her B se konnten wi kei e Aktivitat zwischen den Nerve enden im Cortischen Orga beobachten ATP-ase w jedoch im yn ptischen Spalt zwischen Nerven zellen und Haarzellen orhanden

Die En ymaktivit t war analog der Aktivitat im Gehirn ert lit Wi m Gehirn die Kap lla en in ihrer Basalm mbra in Form von ATP-ase aufwe sen, lches besonders empfindlich gegen Vorfixierung mit Glutarald hyd t so fanden wir das da Spiralgef unter dem Cortischen Organ i seiner Basal membran die gleiche Art von ATP-ase-Aktivitat zeigt Zwischen den Gehirnge fassen und Nervenzellen befinden ich Stutz (Glia) zellen w lche ATP-ase-Akti itat zeigen Die Nervenzellen selbst haben jedoch k i Enzym ihrer Ober flache Wir beobachteten dass di Stutzzellen im Cort schen Organ ebenfall Enzymaktivitat haben und dass sich diese Zellen in in W se zwischen Gef

und Haarzellen verteilen welche an das topographische Verhältniss zwischen Nervenzellen Stützzellen und Gefässen im Gehirn erinnert

Die unbekleideten Nervenfasern welche quer durch den Cortischen Gang laufen zeigten Enzymaktivität Die Anwesenheit des Enzyms auf diesen Fasern ist *ein indirektes Zeugnis dafür dass die Flüssigkeit im Gang* eine relativ niedrige Kaliumkonzentration hat, da der Hauptenergieverbrauch von Nervenzellen auf den Kaliumrücktransport gegen einen Konzentrationsunterschied nach der Depolarisation gerichtet ist Sollte die Flüssigkeit welche diese Fasern umgibt dieselbe Kaliumkonzentration wie die Endolymphe haben so wäre keine ATP-ase für den Aktivtransport notwendig.

ATP-ase Aktivität war an der Seite der Haarzellen welche mit der Corti lymphe" in Kontakt steht nicht vorhanden Ein Zylinder von komplexen Membranen mit zahlreichen Mitochondrien bestückt befindet sich nahe der Haarzellenoberfläche in dieser Gegend Abwesenheit von ATP ase in diesem Teil der Haarzellenplasmamembran legt den Schluss nahe das *dieses spe ielle* Membransystem eine andere Funktion hat als den Transport von Materialien von oder zu der „Cortischen Lymphe" Es ist möglich dass diese komplizierte Struktur für den Transport innerhalb der Haarzellen notwendig ist Diese Art von chemischem Transport könnte mit der Informationsübertragung vom Haarfortsatz zur Nervenendung zusammenhängen

REFERENCES

Baker, P F 1966: The odium pump. *Fnd vo r 23, 166.*

Barn ett R. J 1962 The fin tructural localizati of cet cholinesterase t the my eural ju ction *J Cell Bi l 12* 247

— 1964 Localization of enzymatic cti ity t th fin structural level *J Roy Micr Soc 83 143*

Bursto e, M S., 1962 *En ym hi toch mistry* Ac d mi Press, New Y rk nd London.

Engstrom H Ades, H W and Hawki s, J E., Jr 1962 Struct re and function of the sensory hairs of the i n r ea *J Acou Soc Am r 34* 1356

Engstrom H and Wersäll, J 1953 I there a special nutritive cell l r syst m around tl h i cells in th rgan f Corti? *Ann Ot l 62* 37

Hildi g A C., 1952 Studies n the tic l byrinth. I O th rigin and los rti f th tectorial membra *Ann. Ot l 61* 354

Kikuchi, K a d Hildi g, D A 1965 Th developm nt f the rga f Corti in the mouse *A ta Otolaryng* (Stockh.) *60* 207

— 1966 Th piral essel a d stria ascularis i Shak 1 mice Electron microscopic nd histochemical bser ti ns. *Acta Ot laryng* (Stockh) *63* 395

Lawrence, M 1966 Effects f interf rence with terminal blood supply o the organ of Corti *Laryngos pe 76* 1318

Marchesi, V T nd B rnett R. J 1964 Loc li ati of nucleosid phosphat se acti ity in different t pes f mall blood essel *J Ultra t u t R s 10* 103

M rchesi, V T., Sears, M L., d Barn ett R J 1964 Electro microscopic studies f nucleosid phosphatase acti ity i blood essel and glia of the reti a *Invest Ophthal* 3 1

Mizukoshi, O 1966: Pers nal Comm nicati

N k i, Y a d Hilding, D A., 1966: Electro microscopi studi f deposi e triphosphatase ctivity in the tri ascul I d piral lig ment *A t Otolaryng* (Stockh) *62* 411

PADYKULA, H. A., and HERMAN, E., 1955 Factors affecti g the activity f adenosinetri phosphat se nd other phosphat ses measured by histochemical tech iques. J Histochem. Cytochem 3 161

PEARCE, A. G E., 1961 Histochemistry Theoretical and pplied J & A Churchill, Ltd.

RA CH, S 1964 Biochemi der Hörorgans. Thieme, St tig rt

SABATINI, D D MILLER, F nd BARRNETT R J 1964 Aldehyde fixation f mor phological nd enzyme histochemic l studies w th electron microscope J Histoch m Cytochem 12, 57

SCHWARZ, A., BACHELARD, H S., nd M I w ry, H., 1962 The sodi m timulated ade osine triphosphatase activity nd other properties of cerebral microsomal fractions and subfractions. Bioch m J 84 626

SIEKEVITZ, D LOW H ERNSTER, L., nd LI DBERG, O 1958 On possibl mecha ism f the adenosinetriphosphatase f li er mitochondria. Biochim Biophys. Act 29 378

SKOU J C., 1962 Preparation from the mammalia brain and kidney of the enzyme ystem involved in the activ transport f sodi m nd potassi m Biochim Bi phy Act 58 314.

TASAKI, I., D VIS, H., nd ELDREDGE, D H., 1954 Exploratio of cochlear potential in guinea pigs with microelectrode J Acous. Soc Amer 26 765.

TONNDORF J DUVALL, A J nd REN KA J P 1962 Permeability f i tracochlea membranes t ario ital stai s. Ann. Otol 71 801

TORACK, R. M., 1965 Adenosinetriphosphatase cti ity in rat brain following differential fixation with formaldehyde, gl t raldehyde nd hydro yadipaldehyde J Histochem. Cytochem 13, 191

TORACK, R M and BA RNETT R J 1963 Nucleotid phosphat se activity i mem branous fine structures of neuro d glia. J Histochem Cytochem., 11 763

VINN KOV Y A and TITOVA, L. K., 1964 The rgan of Corti, it hist physiology and histochemistry Consultants Bureau, New York.

WACHSTEIN, M., and MEISEL, E., 1957 Histochemistry f hepatic phosphatases t physiologic pH with pecial reference to the demonstratio f bile canaliculi Amer J Cli Path., 27 13.

Th Otol ryngology Secti and Otologic
Research Laboratory Yal U iversity
School of Medicin N w Haven Conn
U.S.A

Received March 8 1967

Acta oto-laryngologica 64 192 199 (1967)

PROSTHETIC RECONSTRUCTION OF EXTERNAL EAR DEFECTS

P. Vang Jensen and K. Terkildsen
Copenhagen Denmark

From the Ear Nose and Throat Department of Rigshospitalet (Head Prof H K Kristensen) University Clinic Copenhagen

After a discussion of indications for surgical and/or prosthetic reconstruction of ear defects a detailed description is given of the technical procedure we use in the fabrication of the latex type prosthesis. We have treated 19 patients and in 13 the treatment was a complete success in spite of a high proportion of children in the series. Three of the unsuccessful cases were young women that preferred to hide the defect with a suitable hairdo

The results of surgical procedures for reconstruction of major ear defects are less than ideal even in the best hands and it is a common experience that a prosthesis often produces the most gratifying result

Prosthetic treatment is a borderline field and most surgeons have a rather vague knowledge about modern prosthetic work For natural reasons it is the surgeon who decides which treatment should be preferred in the individual case There seems to be a need for discussion of prosthetic problems in the otological literature

Defects of the pinna result in a striking asymmetry of the face which invariably attracts attention Very few patients are psychically robust enough to tolerate constant attention to such a defect and often the need for treatment becomes imperative

Surgical reconstruction may accomplish relatively satisfactory imitations of the ear when seen from the side It is difficult however to obtain a sufficient degree of protrusion, and in most instances the facial asymmetry persists when seen in a frontal view (Fig 3)

Bulbulian (1939) introduced prevulcanized latex for the fabrication of somato-prostheses According to present standards this material has several shortcomings especially regarding its durability Nevertheless it has proven itself particularly useful for artificial ears because of its low weight, its pliability and the ease of retention

In recent years there has been a steady increase of new materials by means of which it is possible to produce a more permanent prosthesis of satisfactory quality Unfortunately they all suffer from the disadvantage of being solid This will reduce the pliability and the weight is increased to such an extent that retention of the prosthesis becomes a serious

problem. Several ingenious methods have been suggested in order to over come this difficulty (Boenninghaus & Pfütz, 1957 Werner 1963 Dunlon et l 1964 Dubs, 1965)

In this country prevulcanized latex has been used for the reconstruc tion of aural defects since 1951 (Jarby 19₅1) The technique has remained essentially unchanged and shall be described in detail

Plaster of Paris models are made from both aural regions according to ordinary dental prosthetic technique For the shaping of a prosthesis it is great advantage to proceed fr m a uitable ear from a normal person A wax model is obtained of such a donor-ear and fitted to the plaster of Paris model of the aural region This fitting process is a critical point and should be performed with meticulous care aiming at perfect symmetry with the other ear After finishing the fitting and reshaping of the wax ear it is advisable to try it on the patient in order to ascertain the result especially with a view to the positi n and the degree of protrusion. The other half of the final mold can now be completed The wax ear is fixed in its proper position on the ear region model by means of a little molten wax. A thin layer of vaseline is applied to the remainder of the surface in order t facilitate separation A wax mantle i then fitted along the sides in order t hold the plaster which is poured around the ear model to a thickness of 4–5 cm After half an hour th mold can be opened and the w x is washed away with boiling water Finally a 1 cm wide filling canal must be drilled through the scalp part of the mold

The prosthesis itself i made of Revultex (Re ertex Ltd London)

For colouring w use the following colour powders manufactured by I.C.I Ltd. nd in concentrations as indicated

Vulcafor fast red MS, 2%
Waxoline red OS 1%
Vulcafor f st brown B, 1%
Vulcaf r f st blue 5%

Furthermore we employ 20% tit nium dioxide 1% Dispersol is added to ll mixtures as a stabilizer

Basic colour mi tures are made in amounts of 100 ml and will keep indefinitely

A mixture of f i Vulcafor fast red i prepared as follows 2 gm colour powder i mixed carefully with 1 gm Di persol on a glass plate by means f spatula. Distilled water is added dropwise until the consistency is like a soft paste The mixture is then transferred to a bottle containing 100 ml distilled wate and haken thoroughly

The titani m dioxide mixture is prepared from 20 gm titanium dioxide and 1 gm Di persol in th same way

For the fabrication of the prosthesis 100 ml Revultex is put in a glass bottle with firm lid and colours ar added. For Danish conditions we often use 60–120 drops of titanium dioxide and 20–70 drops of Vulcafor



Fig 1 The two parts that constitut the fin l mold for the prosthesis. To the left the aural regi n mod l with filling h l in the center

fast red Finally it is necessary to add as a stabilizer 1 drop of a 20% Vulcastab LW solution in distilled water

After gentle stirring with a glass spatula the mixture is ready

Before use the mold should be moistened by filling it with distilled water which is removed again after about 1 min This procedure will permit the latex mixture to flow freely and fill all spaces The latex should remain in the mold for a period of 0–15 min according to the desired thickness of the membrane The remainder is then poured out and can be returned to the bottle for further use The mold is placed in a thermostat at 70 C for 18–20 hours After adding some talcum powder via the filling hole the prosthesis is removed. The durability can be increased to a con siderable degree by placing it for 8–10 min in a bath consisting of 5 ml bleaching water 3 ml 37% hydrochloric acid and 100 ml distilled water Following this it should be rinsed in water for 24 hours in order to remove all chemicals The bath will not only harden the surface and close small porosities, but the treatment also tends to enhance the skinlike quality of the surface

After finishing it is possible to add colour to the prosthesis from the inside with a small cotton applicator and colour powder in benzine

Retention is achieved by means of a special glue delivered from a factory for medical type adhesive tapes About once a week it should be removed for cleaning With the above-mentioned modification a single prosthesis will last for a period of at least 3 months

Roughly speaking aural defects are of two categories Traumatic or surgical defects which are usually total except for the tragus, and congenital malformations which are partial and very often without a tragus. The first type is ideally suited for prosthetic treatment The presence of a tragus makes it possible to obscure the anterior line of transition between prosthesis and normal skin which usually is a critical point in rendering a prosthesis acceptable from a cosmetic point of view Absence of the pinna furthermore lea es a smoth surface for attachment.

Congenital malformations constitute a highly mixed group If the deformed ear protrudes from the scalp to a normal degree surgical procedures usually produce satisfactory results. In most instances, however there will be more or less flat rudiments unfit for plastic surgical reconstruction Unfortunately the rudiments are often positioned just where the artificial ear should imitate the conchal cavity so that it is impossible to cover the abnormal structures with a natural looking prosthesis. Bulbulian & Litrow (1961) have discussed these problems very adequately They point to the advantage of a tragus when fitting a prosthesis and suggest a method by which the rudimentary aural cartilages after reshaping are transferred to another site in order to constitute a tragus-like structure At the same time rudiments may be removed from places where they are in the way for a proper-fitting prosthesis. All too often prosthetic treatment is not considered until one or several unsuccessful surgical procedures have been performed It would be a great ad antage if in the individual case the patient, the surgeon, and the prosthetist could decide beforehand which treatment should be preferred. If the decision favours a prosthesis all efforts should be directed toward the accomplishment of optimal conditions for a prosthesis and there should be no compromise.

We have treated a total of 10 patients with prosthetic ears. Because of the need for regular renewal of the prosthesis we are in frequent contact with the patients and have a reliable basis for judging the results In 4 patients the defects were of traumatic origin (horse bite, burn, traffic accidents) In one the defect was only partial and treatment proved unsuccessful The other 3 were complete successes One is a city bus driver He has used a prosthesis for 12 years and no one ever notices his defect (Fig. 2.)

The remaining 15 are congenital malformations. One has bilateral defects, th others are unilateral 5 patients have discontinued the prosthetic treatment. 3 of these are young girls They all prefer to hide the d fect with suitable hairdo and feel that the gain obtained by application f the prosthesis does not outweigh the trouble with handling it One was 3-yea old boy to whom we fitted a partial prosthesis, and finally there w a 48-years-old male with a t tal absence of one ear who never got st rted u i g the prosthesis.

Of the 10 patients in this group who use a prosthesis continuously 4 had bee ubmitted t plastic surgical procedures aiming at recon truction of

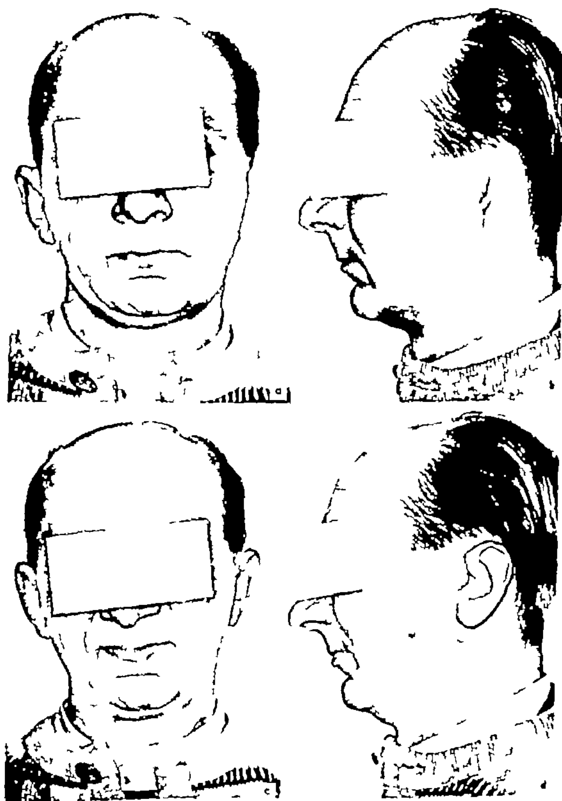


FIG. 2. Patient with traumatic defect. Tragus present. With and without prosthesis.

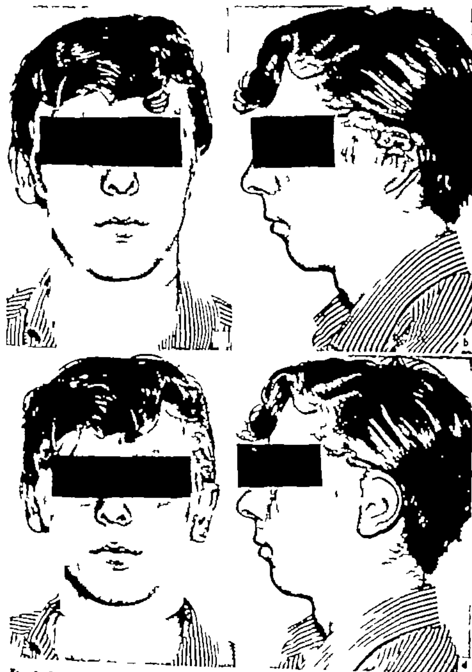


Fig. 3 Patient with unilateral congen t l malf rmat S rgical recon truction n-successf l especially d t lack f protru ion when seen from front l iew There is no tragu and the nterior line of transitio betwe prosthes nd normal skin difficult to obscure

the pinna. Two of these wear a partial prosthesis and in one we finally decided to sacrifice what had been obtained through previous operations and instead an imitation of the tragus was reconstructed which resulted in a considerable cosmetic improvement

Four patients were children in the age from 4–7 years at the time when the treatment was initiated At this age the parents attitude and motivation is a decisive factor Most of the children are now old enough to give an opinion of their own and there is no doubt that the treatment is a success. A fifth child in which the prosthetic treatment was discontinued has been mentioned above

In the course of time our strategy in patients with aural defects has changed gradually from a quite active plastic surgical attitude towards dominance of prosthetic treatment. Surgery is advised only when the rudiments are large enough to offer good possibilities for sufficient protrusion of the reconstructed ear Our results give evidence of the difficulties in fitting a prosthesis if the surgical reconstruction finally proves to be unsatisfactory Quite naturally the patient and the surgeon are reluctant in a decision that involves a lifelong dependence on a prosthesis In cases of doubt we prefer to give the patient the experience of wearing a prosthesis for a while If the trial is a success it is much easier to decide for those surgical procedures that might be necessary in order to improve conditions for a final prosthesis.

In children there is no doubt that surgery should be postponed till immediately before puberty in order to avoid those changes that result from natural growth This applies also to patients with concomitant unilateral atresia of the meatus, where reconstruction is indicated in order to improve hearing Normal hearing in one ear is adequate for normal development and eventual surgery can for technical reasons with advantage be postponed until the mastoid becomes completely pneumatized In patients with bilateral atresia the situation, naturally is entirely different

ZUSAMMENFASSUNG

Die Indikationsstellung für chirurgische autoplastische Formung sowie ein Prothesen Verfahren bei Wiederherstellung des äusseren Ohres werden diskutiert Unsere technische Methodik bei der Herstellung von Latex-Ohrepithesen wird vollständig beschrieben Die Epithesen Behandlung war in 13 von 19 Patienten erfolgreich 3 Frauen gehörten zu der erfolglosen Gruppe

REFERENCES

BOENNINGHAUS, VON H-G and PRUTZ, E., 1957: Zweiteilig Ohrprothese n h Verlust d Ohrmusch l Z. Laryng Rhin l Otol 36 422.

BULBULIAN A. H 1939 An improved techn for prosthetic restorati n of f c l defects by use of a latex compound Proc Staff Meet M y Clin c 14 433

Beimellan, A. H., and Litzow T J 1961 Congenit l malformatio f the ear- Prosthetic reconstruction in combination with plastic surgical proced re *Proc Staff Meet Mayo Cl ic* 36 429.

Drum, ov R., 1965 Eine neue Befestigungsmethode für Ohrmuschel-Epithesen. *Pract Otorhinolaryng* (Basel) 27 172.

Dunton, E., T man, G Lewin, S., d Panzarwski, J 1964: A compromise pproach t total ear reconstructi n. *Plast Reconst Surg* 31 247

Lime, S., 1951 Protetisk behandling f defekter *Tandlaegebladet* 5, 225

Weimer, ov R 1963 Über modifiziert Befestigungsverfahre on Kunstst ff-Ob epithesen nach Ombreda ne-Plastiken. *Z Laryng Rhi ol Otol.*, 42 533

E.N.T Dept Rigshospit let
Blegdamsvej 9
Copenhage Denmark

Received January 12 1967

THE STERNOHYOID MUSCLE DURING PHONATION

Electromyographic Studies

M. HIRANO,[1] Y. KOIKE[2] and H. VON LEDEN
Los Angeles, Calif., U.S.A.

From the Institute of Laryngology and Voice Disorders and the University of Southern California, Los Angeles

Electrical activity of the sternohyoid muscle was studied in three normal male adults and related to phonic functions. The results are summarized as follows. (1) No systematic relationship was found between muscular activity and different vowels. (2) Electrical activity of the sternohyoid muscle was greater at lower and higher pitches and smaller in the middle range. (3) Electrical activity of the sternohyoid muscle increased with increasing volume of voice at each pitch.

Although the past two decades have produced valuable information about the electrical activity of the intrinsic laryngeal muscles in relation to phonation, we know little about the action of the extrinsic laryngeal muscles during voice production. Clinically, there is no doubt that the extrinsic laryngeal muscles play some role in the adjustment of the vocal cords during phonation at various pitches and volumes. Sonninen (1956) made important contributions to the understanding of the role of the extrinsic laryngeal muscles, especially the sternothyroid muscle, during singing by clinical and roentgenological studies. Fink, Basek & Epanchin (1956), Zenker & Zenker (1960), Faaborg-Andersen & Sonninen (1960), Arnold (1961), and Kimura (1961) investigated the electrical activities of some of the extrinsic laryngeal muscles; however, detailed action patterns of these muscles during various changes of voice remain in doubt.

An electromyographic investigation is especially advantageous because it measures the role of each individual muscle in the composite adjustments of the vocal cords and the resonance cavity which represent an integrated result of multiple muscle contraction. As the first in a series of investigations, we have studied the electrical activity of the sternohyoid muscle in its relation to the pitch and volume of voice.

This research was supported by USPHS Research Grant NB 06670 from the National Institute of Neurological Diseases and Blindness.

[1] On leave of absence from the Department of Otolaryngology, Kurume University, Japan.

[2] On leave of absence from the Department of Otolaryngology, Kyoto University, Japan.

Technique of Investigation

Three male adults served as subjects for the present study. All subjects had some training in singing though none is a professional singer.

A concentric needle electrode was inserted into the sternohyoid muscle at the level of the thyroid cartilage where this muscle was easily palpated. The electrode was connected to a high gain amplifier (Sanborn Model 350) which was hooked up to one channel of a galvanic poly beam recorder (Sanborn, Model 508). A contact microphone was placed on the skin of the neck in front of the trachea and the voice signal was recorded on another channel of the poly beam recorder. Simultaneously the voice signal was picked up at the front of the mouth by a condensor microphone (Sony C-37A) and recorded on a tape recorder (Sony 777) for the purpose of identification and acoustic analysis. Throughout the experiments, both electrical activity and voice signal were displayed on a cathode ray oscilloscope (Tektronix, type 565) for the purpose of visual observation.

Evaluation of Muscular Activity

With increasing contraction power of a muscle the rate and number of single motor unit potentials increase; finally it becomes impossible to distinguish the potentials of each motor unit, resulting in an interference pattern. We realize that the maximum amplitude of the interference potentials obtained with a concentric needle electrode does not show a linear relationship to the strength of muscle contraction, yet this measurement has been employed extensively, especially in small muscles, in order to compare the power of contraction, as long as the electrode remains in the same position.

In the present study most recordings of electrical activity showed interference patterns. Therefore the maximum amplitude of interference potentials was measured to compare the strengths of muscle contraction qualitatively within the same subject. It should be emphasized that this procedure does not imply quantitative comparisons of contraction power of the muscle. In other words, a contraction measuring 200 μV does not denote twice the strength of a contraction measuring 100 μV. In each experiment we devoted special attention to the fixation of the electrode in the same location for the duration of the test.

RESULTS

Electrical activity of the sternohyoid muscle in relation to vowels

Since the extrinsic laryngeal muscles participate in articulation, we started our investigation by measuring the effect of different vowels on the electrical activity of the sternohyoid muscle. Five vowels, a, ɔ, a and i were tested at various pitches and volumes. There were no

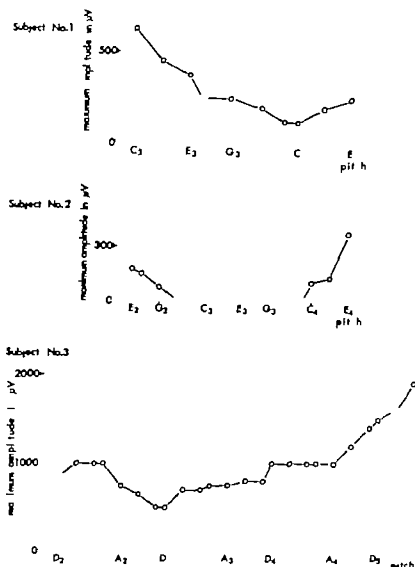


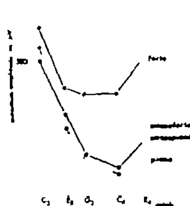
FIG. 1 Maximum mplitude f lectrical ctivity of the st rn hyoid muscle d ring the inging of a mu ical scale with consistent sound olume

systematic relationships between the maximum amplitude of electrical activity and the different vowels any variations in electrical activity occurred on the basis of subject, pitch and/or volume of voice When attention was directed to the relationship of electrical activity in the sternohyoid muscle to the pitch or volume of voice similar tendencies were found for every vowel in all subjects.

In this paper we confine our description chiefly to the vowel *a* because we found no essential differences for the other vowels in relation to pitch or volume

Electrical activity of the sternohyoid muscle in relation to pitch

In order to study the relationship between electrical activity of the sternohyoid muscle and pitch of voice two sets of experiments were per



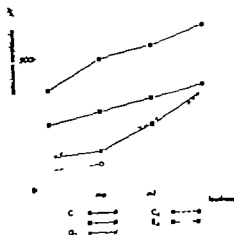


Fig. 2 Fig. 3

Fig. 2. Maximum amplitude of electrical activity of the sternohyoid muscle related to vocal pitch at various sound volumes of voice.

Fig. 3. Maximum amplitude of electrical activity of the sternohyoid muscle related to volume of voice at various pitches.

formed in each subject. First, the subjects were instructed to sing an ascending musical scale with an effort to keep the volume constant the amplitude of the voice signal was displayed on the cathode ray oscilloscope The change in register occurred smoothly without any "break Second, the subjects sang the vowel *a* at four volumes, piano, mezzopiano, mezzoforte and forte, at three to five selected pitches within their musical vocal range

Fig. 1 shows the relationship between the electrical activity of the sternohyoid muscle and the pitch during the singing of a musical scale with consistent volume Though there were considerable differences of absolute voltage the same tendency was found in all of the three subjects The electrical activity was most pronounced in both lower and higher ranges and was less marked at the middle portion of the scale, generally showing a V-shaped curve The pitches associated with minimum electrical activity differed from subject to subject. The minimum electric activity occurred usually in the middle register although we observed no definite relationship between the electrical potential and different registers.

Results of the second procedure in one subject (No. 1) are presented in Fig. 2, which confirms the results of the first experiment. The other two subjects showed very similar trends.

Electrical activity of the sternohyoid muscle in relation to volume

The second procedure of our investigation also revealed a relationship between the electrical activity of the sternohyoid muscle and the volume of voice Fig. 3, a modification of Fig. 2, shows that the electrical activity of

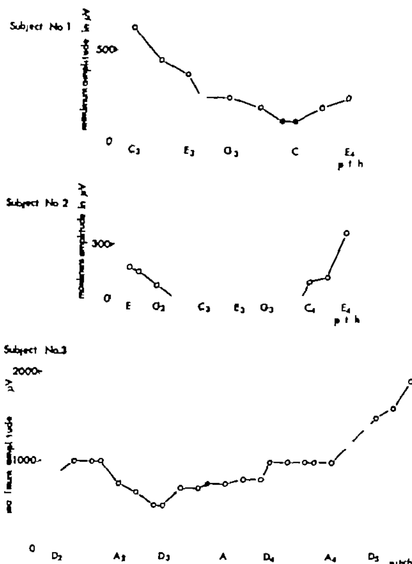


Fig. 1 Maximum amplitude of electrical activity of the sternohyoid muscle during the singing of a musical scale with constant sound volume

systematic relationships between the maximum amplitude of electrical activity and the different vowels any variations in electrical activity occurred on the basis of subject pitch and/or volume of voice When attention was directed to the relationship of electrical activity in the sternohyoid muscle to the pitch or volume of voice similar tendencies were found for every vowel in all subjects.

In this paper we confine our description chiefly to the vowel *a* because we found no essential differences for the other vowels in relation to pitch or volume

Electrical activity of the sternohyoid muscle in relation to pitch

In order to study the relationship between electrical activity of the sternohyoid muscle and pitch of voice two sets of experiments were per

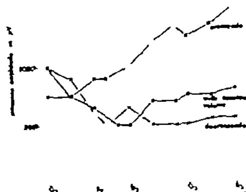


Fig. 5. Maximum amplitude of electrical activity of the sternohyoid muscle during the singing of musical scale with three different intensity variations.

the sternohyoid muscle increases with increasing volume of voice at any pitch. Similar results were obtained in the other two subjects, except for vowel ɔ of one subject (No 2) where the electrical activity appeared smaller in mezzoforte than in piano and mezzopiano Fig. 4 demonstrates the actual recordings.

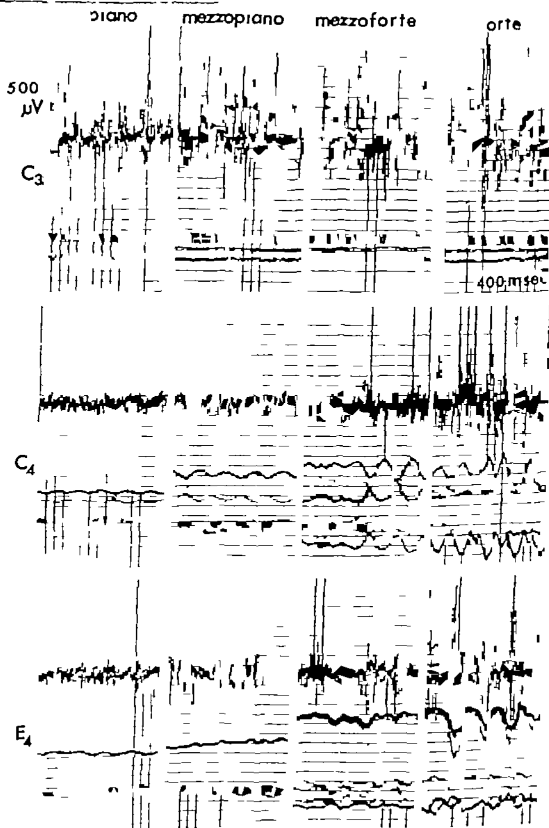
Electrical activity of the sternohyoid muscle in relation to combined change in pitch and volume

In order to determine the influence of combined changes in pitch and volume of voice upon the electrical activity of the sternohyoid muscle the subject sang a musical scale in three different ways (1) with an effort to keep the volume constant, (2) with increasing volume—crescendo, and (3) with decreasing volume—decrescendo. Fig 5 demonstrates the results obtained in one of the subjects (No. 3) A comparison of the changes in the electrical activity of the sternohyoid muscle during a crescendo or decrescendo scale with changes during a scale at consistent volume clearly demonstrates that electrical activity is influenced by both pitch and volume of voice.

DISCUSSION

The extrinsic laryngeal muscles are concerned with both vocal cord adjustment and with the shape of the resonance cavity The action of these muscles during phonic function differs from subject to subject depending upon the vocal habits or vocal training. Therefore, it is difficult to draw far-reaching general conclusions from the results of the present investigation They do, however suggest some functions of the sternohyoid muscle concerned with the regulation of pitch and volume of voice

The primary function of the sternohyoid muscle is to draw the hyoid



F o. 4 Actl n potentlals of the stern hy id muscl t rl plt hes nd lumes f oice

REFERENCES

ARNOLD, G. E., 1961: Physiology and pathology of the cricothyroid muscle. *Laryngoscope 71* 687

DEDO, H., and D... ER, E., 1966 The volume conduction of motor unit potentials. *Electroenceph. Clin. Neurophysiol. 1 1 20* 606.

FAABORG-ANDERSEN, K., 1957 Electromyographic investigation of intrinsic laryngeal muscles in humans. *Acta Physiol Scand., 41* Suppl. 140

FAABORG-ANDERSEN, K., and SONNINEN, A. 1960 The function of the intrinsic laryngeal muscles at different pitch. *Acta Otolaryng* (Stockh.) *51* 89

FINK, B. R., BASEK, M. and EPANCHIN, V. 1956 The mechanism of opening of the human larynx. *Laryngoscope 66* 410

FAULCONER, R. and HOFER, G. 1963 *Tomographische Studien zur Funktion des menschlichen Kehlkopfes. Folia Phoniat* (Basel) *17* 83

GRANT, J. C. B., and SMITH, C. G. 1953 *Morris' Human Anatomy*. McGraw-Hill Co., New York.

ISSHIKI, N. 1959 Regulatory mechanism of the pitch and volume of voice. *Otorhinolaryng Clinic* Kyoto, 52, 1065

— 1964 Regulatory mechanism of voice intensity variation. *J. Speech Hearing Res.*, 7 17

KENYON, E. M. 1922 Significance of the extrinsic musculature of the larynx. *J.A.M.A.* 79, 428.

KOYAMA, N. 1961 Electromyographic study of the extrinsic muscle of the larynx. *Otorhinolaryng Clinic* Kyoto, 54 481

LUCHSINGER, R., and ARNOLD, G. E., 1965 *Voice-Speech-Language*. Wadsworth Co., Belmont, Calif.

Mayo Clinic, 1956 *Clinical examination in neurology*. Saunders Co., Philadelphia.

SONNINEN, A. A., 1956 The role of the external laryngeal muscles in length-adjustment of the vocal cords in singing. *Acta Otolaryng* (Stockh.) S ppl 130

SPALTEHOLZ, W. 1913 *Hand-Atlas of Human Anatomy* Vol. I Lippincott Co. Philadelphia

VENNARD, W. 1964 *Singing. The Mechanism and the Technic*. Edwards Brothers, Inc. Ann Arbor, Mich.

ZENKER, W. and ZENKER, A. 1960 Über die Regelung der Stimmlippenspannung durch von aussen eingreifende Mechanismen. *Folia Phoniat 12*, 1

H. von Leden, M.D., The Institute of Laryngology and Voice Disorders, 11600 Wilshire Blvd. Los Angeles, Calif. U.S.A.

Received March 31, 1967

bone downward In combination with the other neck muscles, this muscle can participate in positioning the larynx and mandible and presumably to some extent indirectly in vocal cord adjustment. In terms of the laryngeal position the two sternohyoid muscles assist in drawing the larynx downward or in preventing its elevation

Although there is no specific relationship between the position of the larynx and the pitch of the voice it is generally accepted that in the untrained voice the larynx is elevated with a rise in pitch while the well-trained singer has learned to reduce the movements of the larynx during changes in pitch In the present investigation, greater activity of the sternohyoid muscle at lower pitches appears to be related chiefly to the low position of the larynx On the basis of electromyographic studies, Zenker & Zenker (1960) reported that in the usual speaking range the larynx is placed near its static rest position lowering or elevation of vocal pitch is accompanied by increased activity of shortening or tensor elements. Our findings of reduced activity at middle pitches support their description. The greater activity at higher pitches indicates that the subjects made some effort to minimize the elevation of the larynx, as they had had some vocal training There are other possible interpretations about alterations of electrical activity in the sternohyoid muscle with changing pitch, which are concerned with changes in register or in the resonance cavity Further investigations are in process to present a conclusive answer

With reference to the regulatory mechanism of voice intensity Isshiki (1964) concluded that at very low pitches, the glottal resistance is dominant in controlling intensity while at high pitch the intensity is controlled almost entirely by the flow rate In the present investigation the muscular activity of the sternohyoid muscle increased with increasing volume of voice at any pitch Though this muscle is not essential to phonation, the data suggests that this muscle could play a supplemental role in the regulation of volume. Further investigations will prove whether the sternohyoid muscle participates in adjusting glottal resistance, in controlling air flow in altering the shape of the resonance cavity or style of phonation during changes in volume, or whether the increased muscular activity during louder voice is an unnecessary effort related to poor vocal technique

ZUSAMMENFASSUNG

Wir haben die elektrische Aktivität des M sternohyoideus an drei normalen männlichen Versuchspersonen untersucht wobei besonders die phonische Funktion in Betracht gezogen wurde Die Versuchsergebnisse in kurzem sind folgendermassen 1 Es besteht keine gesetzmässige Beziehung zwischen der Aktivität des M. sternohyoideus und der Vokalbildung 2 Die Aktivität des M sternohyoideus ist grösser bei tiefen und hohen Tönen dagegen minimal in der mittleren Tonspanne 3 Die Aktivität des M. sternohyoideus verstärkt sich mit zunehmender Stimmintensität bei jeder Frequenz.

The aim of the present investigation was to analyse the permeability and resistance of the maxillary ostium in acute rhinitis, and to compare the results with those in acute and chronic sinusitis. The question of whether acute sinusitis developing from acute rhinitis usually starts with an ostial obstruction or with swelling of the antral mucosa was also studied

MATERIAL AND METHODS

The material consisted of 23 adult persons with symptoms of acute rhinitis which had been present for 1–10 days. Twenty of them were young men doing their first military service and three were patients at the Oto-Laryngological Out-patients Clinic. Both maxillary sinuses were investigated in all subjects except two altogether 44 maxillary sinuses were thus examined. A red swollen mucosa with serous or sero-mucous secre tion was present in most subjects, but in no case was there pain, fever or accumulation of pus in the nose Roentgenography of the paranasal sinuses was performed about one hour before investigation of the ostial permeability and resistance

The permeability of th maxillary ostium was studied by simultaneous recording of the nasal and antral pressures during respiration blowing and sniffing (Drettner 1965 *a*) A spray of Xylocain® without exadrine was used for anaesthesia. For measurement of the resistance of the ostium, a bottle containing irrigation fluid connected to the puncture needle was raised by an electrically driven elevator (Drettner 1965 *b*) The time required for filling of a normal maxillary sinus with irrigation fluid cor responds to elevation of the bottle about 16 cm above the level of the ostium. Higer elevation is required when the resistance of the maxillary ostium is increased. The error of the measurements in pathological sinuses is less than in normal sinuses owing to the smaller capacity of the sinus and compression of the ai in the sinus by the irrigation fluid during the levation of the bottle wh n the ostium is obstructed

RESULTS

Th duration of the rhinitis in the 23 subjects is shown in Table 1

Table 2 hows the di tribution in per cent of the different alterations in ostial *permeability* For comparison the results of previou studies of ostial permeability in 74 sinuses with acute inusitis and in 35 with chronic sinusiti (Drettner 1960) re given in the same table. Only 8 of the 44 sinuses in ases with acute rhinitis showed a patent ostium In 20 the ost um wa *obstructed* even during blowing *and sniffing*, and the rest showed some other kind of altered ostial permeability The frequency of ltered permeability was only lightly below that in acute sinusitis. An ostium bstructed during blowing and sniffing was found in 45% in acute

Acta oto-laryngologica 64 508–513 (1967)

THE BORDERLINE BETWEEN ACUTE RHINITIS AND SINUSITIS

B Drettner and C. E Lindholm
Uppsala Sweden

From the Department of Otolaryngology (Head Prof A Sjöberg)
University Hospital Uppsala

The permeability and resistance of the maxillary ostium was investigated in 44 maxillary sinuses in 23 patients with acute rhinitis which had been present for 1–10 days Only 8 of the sinuses had a patent ostium 16 had an obstruction which was overcome during blowing or sniffing, and the rest were obstructed during blowing and sniffing. Ostial obstruction showed a positive correlation to pathological roentgenography of the sinus, but the former was somewhat more common It seems therefore probable that acute sinusitis usually starts with an ostial obstruction Antral irrigation yielded a small quantity of mucus from 6 of the sinuses, but otherwise nothing The median ostial resistance was only slightly below that in acute sinusitis.

Acute sinusitis is usually a complication of acute rhinitis, where the infection propagates through the ostia of the paranasal sinuses The development of acute sinusitis may thus be dependent both on the duration of the rhinitis and on the permeability of the ostia of the sinuses during the course of the rhinitis. The title of this article refers to both these aspects

Several studies of the permeability of the maxillary ostium have shown that this permeability is usually reduced in acute sinusitis (Döderlein 1932 Schmücker 1932 Proetz, 1932 Kerekes, 1934 O Malley 1935 Metz, 1939 Stachurski 1950 Flottes *et al* 1960 Drettner 1965 *a*) The question of whether this ostial obstruction is the origin of the sinusitis or only a secondary result of the swelling of the antral mucosa has not been answered however It is surprising that no investigations of the ostial permeability during acute rhinitis appear to have been reported in the literature

Metz (1939) discussed the question of whether the permeability of the maxillary ostium might be disturbed during acute and chronic rhinitis. In his series of 261 punctures in 133 patients the respiratory fluctuations of the antral pressure were mostly normal in cases with normal roentgenography of the maxillary sinus, and reduced in cases with pathological roentgenography His series was not classified as regards diagnosis, but he supposed that some of the cases with normal roentgenography might have rhinitis. He also discussed the possibility that the cocaine used for anaesthesia might have improved the ostial permeability



Fig. 1 The median pressure required t... ercome the osti l resi tance i acut rhinitis, and acute and chronic ... nusitis.

rhinitis, in 60% in acute sinusitis and in all of the investigated patients with chronic sinusitis.

Alterations in ostial permeability were more common and more pronounced in the cases where roentgenography showed pathological changes (mucosal swelling, polyps or blurring) than in those with normal roentgenography (Table 3) The correlation between ostial obstruction and pathological roentgenography was statistically significant ($\chi^2 = 9.01$ $P <$ 0.01)

The frequency of both pathological roentgenography and ostial obstruction were somewhat greater when the rhinitis had lasted 5–10 days than with a duration of 1–4 days (Table 4) The frequencies of pathological roentgenography in these two groups were 30% and 41% respectively while the corresponding figures for obstructed ostium were 37% and 59% These differences were not statistically ignificant, however

Antral irrigation gave a small quantity of mucus in 6 of th sinuses In the rest the irrigation ga e no result The pressure required to overcome the ostial resistan e among the 44 examined inuses varied between 8 cm H_2O and 100 cm H O the latter being the highest pressure that could be obtained with the elevator The median was 28.5 *cm* H O (Fig. 1) In an other seri s of 43 antral lavages for acute sinu itis and 34 for chronic sinusitis the corresponding median pressures were 34 *cm* H_2O and 86 cm H_2O respectively (Drettner 1066)

DISCUSSION

A frequency f 34% with pathological roentgenography of the paranasal sinuses n a series of cases with acute rhinitis may appear high *Most of* the subject were men in military service and it cannot be excluded that sim lar studies among person in civil life might gi e different results. Hueh (1949) found that 8% of a series of 4682 patients n t uspected of hav ng sinusitis showed blurring of the inuses on roentgenography among those pati nt with diseases of the lower respiratory organ the corresponding figure w 20%

TABLE 1 *The duration of symptoms in 23 patients with acute rhinitis*

Duration days	1	2	3	4	5	6	7	8	9	10
Number of patients	1	2	5	6	1	2	5			1

TABLE 2 *The occurrence (in per cent) of various alterations in permeability of the maxillary ostium in acute rhinitis and acute and chronic sinusitis*

Maxillary ostium	Acute rhinitis %	Acute sinusitis %	Chronic sinusitis %
Patent	18	8	
Partially obstructed	14	12	
Patent after blowing or sniffing	16	18	
Valve	7	12	
Obstructed on blowing and sniffing	45	50	100

TABLE 3 *The relation between alterations in ostial permeability and the result of roentgenography in 44 maxillary sinuses*

	Patent	Partially obstructed	Patent after blowing or sniffing	Valve	Obstructed on blowing and sniffing
Normal roentgenography	7	4	7	3	8
Pathological roentgenography	1	2			12

TABLE 4 *The number of maxillary sinuses with pathological roentgenography and obstructed ostium on blowing and sniffing in relation to the duration of the acute rhinitis*

Duration of rhinitis	Number of maxillary sinuses	Pathological roentgenography	Obstructed maxillary ostium
1–4 days	27	8 (30 %)	10 (37 %)
5–10 days	17	7 (41 %)	10 (59 %)
Total	44	15 (34 %)	20 (45 %)

— 1964. The maxillary ostium in sinusitis. *Eye Ear Nose Throat Monthly 43*, 64.
FLOTTES, L., CLERC, P., R.... R. and DEVILLA, F. 1960. *La physiologie des sinus, ses applications cliniques et thérapeutiques*. Librairie Arnette, Paris.
KRIEGER, G., 1934. Nasennebenhöhlen und Atemmechanismus. *Acta Otolaryng* (Stockh.) *21*, 438.
O'MALLEY, J. F. 1925. Ventilation of the nose and accessory sinuses. *J. Laryng. 50*, 389.
METZ, O., 1939. Undersøgelser over respirati... stryksvingningerne i sinus maxillaris. *Nord. Med. 2*, 965.
PROETZ, A. W. 1932. Some intimate studies of nasal function: their bearing on diagnosis and treatment. *Ann. Otol. 41*, 125.
SCHNÜCKER, K., 1932. Die manometrische Messung des Luftaustausches in der Kieferhöhle und ihre praktische Bedeutung. *Z. Hals- Nas.- Ohrenheilk. 30*, 633.
S...ZAL, H., 1959. Znaczenie badania drożności... naturalnych dla diagnostyki i terapii schorzeń zatok bocznych nosa. *Otolaryng. Pol. 13*, 103.

Dept. of Otolaryngology
University Hospital
Uppsala 14, Sweden

Received June 13, 1967

Irrigation of the maxillary sinus gave a negative result in all except 6 sinuses from which a small quantity of mucus was obtained Positive roentgenography of the sinuses during acute rhinitis therefore does not mean from a clinical point of view the presence of a sinusitis requiring irrigation

The maxillary ostium showed reduced patency in most cases of acute rhinitis This result is not quite comparable with that of Metz (1939) since the patients with acute rhinitis in Metz series were not separated from those with other diagnoses, and cocaine was employed as anaesthetic, while the present series consists only of patients with acute rhinitis, principally men in military service and no substance causing shrinkage of the nasal mucosa was used

Ostial obstruction was found to have a positive correlation to swelling of the antral mucosa as seen on roentgenography but the former was more common though not significantly both in cases with short and those with a somewhat longer rhinitis duration This observation favours the theory that acute sinusitis usually starts with an ostial obstruction and that swelling of the antral mucosa appears a little later A slight swelling of the antral mucosa may not be visible roentgenographically however but such minor swelling is probably without importance except when localized to the region of the maxillary ostium

ZUSAMMENFASSUNG

Die Durchlässigkeit und der Widerstand des Kieferhöhlenostiums wurde in 44 Kieferhöhlen bei 23 Patienten 1 bis 10 Tage nach Erkrankung an akuter Rhinitis untersucht Nur 8 Kieferhöhlen hatten ein offenes Ostium, 16 zeigten eine Obstruktion die sich bei Schneuzen oder Schnüffeln öffnen konnte In den restlichen Fällen verblieb das Ostium auch bei Schneuzen und Schnüffeln stets verschlossen Ein Zusammenhang zwischen Röntgenveränderungen in der Kiefer höhle und Obstruktion des Ostiums konnte in einer Reihe von Fällen festgestellt werden Etwas häufiger jedoch war das alleinige Auftreten einer Obstruktion des Ostium. Daraus lässt sich schliessen dass akute Sinusitis wahrscheinlich mit einer Obstruktion des Ostiums eingeleitet wird Kieferhöhlenspülungen ergaben nur bei 6 Kieferhöhlen etwas Schleim Der mediane Ostiumwiderstand war un beträchtlich geringer als bei akuter Sinusitis.

REFERENCES

BUCH A 1949: Non-di gn sed maxill ry sinu iti *Acta Otol ryng* (Stockh.) S ppl 7

DÖDERLEIN W 1932. Experiment ll Untersuch gen zu Phy i logi der Nasen- und Mundatmung und über die phy i l gisch Bed t ng der Nase nebenhohlen *Z Hal Nasen Ohrenheilk* 30 459

DRETTNER, B 1965 a: Th permeability f th m ill ry o ti m *A ta Otolaryng* (Stockh.) 60 304

— 1965 b Measurement f the resist nce f the m xillary osti m. *A ta Otolaryng* (Stockh) 60 499

— 1960 The maxillary ostium in sinusitis. *Eye Ear Nose Throat Monthly* 45 66.
Flottes, L., Clerc, P Riu R., nd Devilla, F 1960 *La physiologi des sinus, se applications cliniques t thérapeutiqu s.* Librairie Arnette, Paris.
Kramer, G., 1934 Nasennebenhöhlen und Atemmechanismus. *Act Otolaryng* (Stockh.) 21, 438.
O'Malley, J F 1935 Ventilation f the nose and accessory sin ses. *J Laryng* 50 339
Metz, O., 1939 Undersøgelser over respirati nstryksvingningerne i l us maxillaris. *Nord. Med* 2, 985.
Proetz, A. W 1932: Some intimate studies of nasal function their bearing on diagnosis and treatment. *Ann Otol* 41 125
Schifferli, K., 1932 Die manometrische Messung des Luftaust sches in der Kieferhöhle nd ihre praktische Bedeutu g. *Z Hal Nase Ohrenheilk.*, 30 638
Szmeja, H., 1959 Znaczenie badania drożności ujść natural ych dl d agnostyki i terapii schorzeń zatok bocznych nosa. *Otolaryng Pol* 13 103.

Dept f Otolaryngology
U iversity Hospital
Uppsal 14 Swed n

Received Jan 13, 1967

Acta oto-laryngologica 64 514–536 (1967)

MALIGNANT TUMOURS OF THE SUBMANDIBULAR GLAND

C. M Eneroth L. Hjertman and G Moberger
Stockholm Sweden

From the Department of Otolaryngology (Head Prof C A Hamberger) and the Institute of Radiopathology (Head Prof L. Santesson) Karolinska Sjukhuset Stockholm

A histological reclassification of 187 primary tumours in the submandibular gland showed a malignant type of tumour in 62 cases. A long-term clinical follow-up study showed that the malignant submandibular tumours have a poor prognosis as compared to corresponding tumours of the parotid gland It has been shown that adenoid cystic carcinomas can be classified into low-grade and high-grade malignant types. In rapidly growing tumours (such as epidermoid carcinoma solid undifferentiated carcinoma and the high grade malignant type of adenoid cystic carcinoma) the poor prognosis is already evident from a 5-year follow-up The present study shows that a much longer follow-up is, on the other hand necessary to disclose the definite prognosis in the more slowly growing low grade malignant type of adenoid cystic carcinoma *In view of the high incidence of malignant tumours of the submandibular* gland there is a great risk that any lump in this region may actually be malignant The importance of the earliest possible radical surgical treatment is stressed

The extensive literature on salivary gland tumours deals chiefly with tumours of the parotid This is natural since about 80 per cent of all such tumours are located in the parotid gland as compared to only about 10 per cent in the submandibular one per cent in the sublingual and about 9 per cent in the small intraoral salivary glands (Seifert 1966) In view *of the anatomical conditions surgical treatment of submandibular-gland* tumours has been much more uniform and free from problems than that of parotid tumours, in which the course of the facial nerve through the gland has produced difficulties in achieving radical surgery Because of this uniform treatment a series of tumours of the submandibular gland is particularly suitable for a correlated clinical histological and anatomical study with the object of investigating the grade of malignancy and prognosis of the various types of tumour

The rarity of tumours of the *submandibular gland* explains why only a few studies specially devoted to histological and clinical aspects of tumours at this site are found in the literature (Dockerty & Mayo, 1942 Beaven 1963 Simons, Beahrs & Woolner 1964) In the publications in which tumours of all major salivary glands are accounted for collectively the

number of submandibular-gland tumours is generally too small to permit any conclusions about the tumours precisely at this site Although the tumours originating in the various salivary glands have essentially similar histological features, the incidence of the different types of tumour and their prognosis are considered to vary with the localization In order to study the histological and clinical features and the prognosis of malignant tumours originating in the submandibular gland, we analyzed a large series of such tumours.

PRESENT SERIES

The investigation is based on an analysis of operated primary tumours of the submandibular gland in 187 patients registered at Radiumhemmet Stockholm, from 1909 to 1965 inclusively Most of the operations were performed at the Department of Otolaryngology Karolinska Sjukhuset.

A new histological examination was made of all the tumours, which were then reclassified according to a modern nomenclature (Foote & Frazell 1954 Eneroth, 1964) This nomenclature is more differentiated than the original one, e.g. Ahlbom (1935) in which several types of tumour were diffusely defined histologically and therefore clinically as well The pre requisites for a histological re-examination existed since the whole tumour material is available in the archives of the Institute of Radiopathology Radiumhemmet, partly as histological slides and partly as paraffin-em bedded material The patients underwent follow up examinations either at Radiumhemmet or at their local hospital, and the data on the clinical course were registered in the records of Radiumhemmet A complete clinical fol-low-up study was possible in all cases, and was continued until 1965

The prognosis of th tumours was investigated by studies of the rate of local recurrence m tasta is, mortality and the survival rate The interval between the first symptoms of the primary tum ur and death in the tumour disease gi es some idea f the grade of malignancy of the tumour The best conception of the prognosi is, however obtained by a tudy of th deter minate survi al rate which is based on the mortality in the tumour disease The determinat survival rate is defined as the relation between th num-ber f survi als and the number of patients f llowed up after correction for th mort lity in intercurrent diseases.

Reclas ification

Hist l gical reclassifi ation of th submandibular tum urs in the 187 patient sh wed a malignant tumour in 62 cases, nd benign one in 125 The ben gn tumours and allied n n-neoplastic conditions have been reported lsewhere (Eneroth & Hjertman, 1966) and will not be discussed i th paper The di tribution of th malignant t mours after reclassifica tion according to the rious types is shown in Tabl 1

TABLE 1 *Malignant tumours of the submandibular gland*

Diagnosis aft reclassification

	No of cases
Adenoid cystic carcinoma	25
Solid undifferentiated carcinoma	15
Epidermoid carcinoma	11
Mucoepidermoid carcinoma	6
Malignant mixed tumour ("Carcinoma in pleomorphic adenoma")	3
Acinic cell carcinoma	1
Malignant lymphoma (lymphosarcoma)	1
T tal	62

The adenoid cystic carcinomas comprise the largest group—40 per cent of all the malignant submandibular tumours. Of particular interest is the high incidence of solid undifferentiated and epidermoid carcinoma in this localization, as well as the occurrence of a case of acinic cell carcinoma.

Adenoid Cystic Carcinoma

On classification after the histological re-examination of the present material tumours from 25 patients were denoted as adenoid cystic carcinomas

Histological features

The histological features of adenoid cystic carcinoma were thoroughly described by Quattlebaum Dockerty & Mayo (1946) and Thackray & Lucas (1960) among others The microscopical picture characteristic of this type of tumour is dominated by epithelial islands of varying size containing mucus masses or usually hyalinized areas These cribriform structures may form small cystic areas, and solid components may be present to a varying degree The tumour is generally incompletely encapsulated and shows infiltrative growth into surrounding salivary gland adipose and nervous tissue The epithelial cells composing the tumour are relatively uniform and uncharacteristic as regards shape size and stainability They are mostly small have little cytoplasm and contain fairly hyperchromatic nuclei without prominent nucleoli

All 25 such tumours in the present series exhibited structures typical of adenoid cystic carcinoma In 20 cases the features were dominated by cribriform partly small-cystic structures (Fig 1) although with more or less extensive solid components in 16 of them (Fig 2) In 5 cases, the solid component predominated (Fig 3) Unquestionable infiltrative growth was



Fig. 1 Adenoid cystic carcinoma Highl d fferent ted, predominant cribriform tru tures Photomicrograph, 400

Fig 2. Adenoid cystic rcinoma. Moderat ly d fferentiated, cribriform nd sol d struc tures Photomicrograph, 400

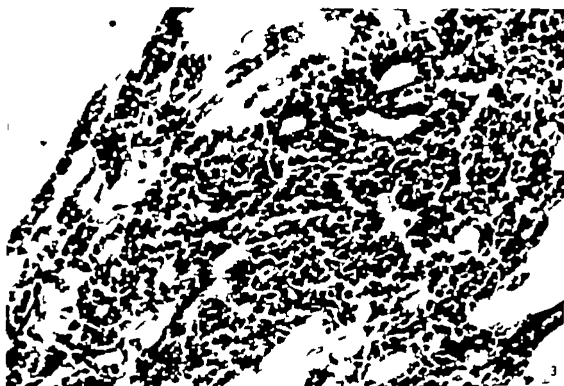


Fig 3 Adenoid cystic carcinoma Poorly differentiated predominant solid structures. Photomicrograph, 400

demonstrable in 24 cases, and was somewhat doubtful in the remaining one Characteristic infiltration of peripheral nerves was present in 3 cases, and distinct vascular invasion in 6

Clinical data

Thirteen of the 25 patients in this group were women and 12 were men The age at primary operation ranged from 37–77 years (mean 56 7) The interval between onset of symptoms of the primary tumour and primary operation was more than 5 years in 9 cases, from 1–5 years in 12 cases, and was less than 1 year in only 4 cases There was a history of pain in 7 of the 25 patients

Follow-up study

Altogether 20 of the 25 patients were operated on in 1927–1961 and were followed up for at least 5 years During the follow up period a local recurrence occurred in 7 cases, in 6 of them within 5 years of primary operation and in 1 case 7 years after it Metastases were demonstrable in 15 of the 20 patients followed up for at least 5 years In 8 cases, the tumour had metastasized to regional cervical lymph nodes and in 7 of them to distant tissues as well In 7 cases there were distant metastases without demonstrable metastasis to regional cervical lymph nodes This implies that distant metastases were present in totally 14 cases The distant metastases

TABLE 2. *5–20 year follow-up study of 20 patients with adenoid cystic carcinoma*

Histological features	No. of patients	Died	
		Of tumour disease	Without signs of tumour diseases
Malignancy grade I Highly differentiated, purely cribriform	4	—	1
Malignancy grade II Medium-highly differentiated, chiefly cribriform, partly solid	12	8	2
Malignancy grade III Poorly differentiated chiefly solid	4	4	—
Total	20	12	3

chiefly involved the lungs (11 cases) but were also found in the brain (2 cases) the liver (2 cases) and the skeleton (3 cases) The interval between detection of the primary tumour and observation of metastatic deposits was often strikingly long. Thus, in 4 of the 7 cases with regional lymph node metastases, the interval was >5 years (1 case >20 years) and in 8 of the 14 with distant metastases this interval was >5 years (3 cases 20 years)

Totally 12 of the 20 patients died of the tumour disease

As previously stated, the tumours are characterized histologically by two structures, i.e. a cribriform component and a solid one. We have considered these structural components to represent different grades of maturity (differentiation) of the tumour tissue the cribriform structure representing high differentiation, and the solid structure poor differentiation

We attempted to grade the malignancy by grouping the tumours on the basis of the relative occurrence of the respective components and of the grade of cellular anaplasia. The tumours were divided into three groups of malignancy. Their distribution in the different groups in the 20 patients who could be followed up for at least 5 years is seen in Table 2.

It is evident from Table 2 that none of the patients with adenoid cystic carcinoma of malignancy grade I died of the tumour disease whereas 8 of the 12 with a tumour of malignancy grade II and all those with a tumour of grade III died of the tumour disease. Three of the 20 patients died of an intercurrent disease one with a tumour of malignancy grade I 3 years after primary operation and 2 with a grade II tumour 2 and 7 years, respectively after it.

Three patients with a tumour of malignancy grade I were alive at the

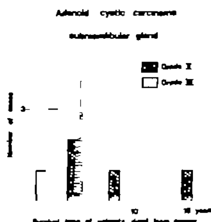


FIG 4

last follow up examination one 6 years after operation and 2 more than 10 years after it Two patients with a tumour of malignancy grade II were also alive on this occasion one 5 years after primary operation and the other 28 years after it although the latter had metastases. The survival time in the 12 patients who died of the tumour disease is also of some prognostic interest (Fig 4)

It can be inferred from Fig 4 that in the 12 patients with adenoid cystic carcinoma who died of the tumour disease the survival time was shortest in those whose tumour was evaluated on the basis of a dominance of solid structures, as poorly differentiated (malignancy grade III) The number of cases is not large enough to permit a statistical analysis The difference in survival time is nevertheless so obvious that it seems justified to state that tumours with solid structures are more malignant than those with a more or less pronounced dominance of cribriform structures.

Table 3 shows the determinate survival rate after 5 10 and 15 years observation in the whole group of patients with adenoid cystic carcinoma, without taking into account the histological subgroups

It is evident from Table 3 that 20 patients had been under observation for 5 years 14 for 10 years and 9 for 15 years Three patients died without signs of the tumour disease The determinate survival rate shows a pronounced decrease in the different groups, i e from 50 per cent in the 5-year group to 25 per cent in the 10 year group and to 0 per cent in the 15-year observation group

Treatment

Seven of the 25 patients with adenoid cystic carcinoma underwent surgical treatment only whereas 18 were treated by a combination of surgery and radiotherapy Surgical treatment consisted of excision of the tumour in 5 cases and of evacuation of the submandibular region in 20 In 7 of the 20 cases, radical neck dissection was also undertaken

TABLE 3 *Adenoid cystic carcinoma of the submandibular gland 5–15 year follow-up study*

Observation period (years)	No. of patients	Died: Of tumour disease	Died: Without signs of tumour disease	Determinate survival rate: No. of patients	No. of survivals	Survival
5	20	9	2	18	9	50.0
10	14	9	2	12	3	25.0
15	9	7	2	7	0	0

In the 18 cases in which irradiation therapy was given it was given both pre and postoperatively in 2 preoperatively only in 1 and postoperatively only in 15 cases.

Mucoepidermoid Carcinoma

On histological re-examination of the present material we found the tumours from 6 patients to show the features described in detail by Stewart, Foote & Becker (1945) as characteristic of mucoepidermoid carcinoma

Histological features

Foot & Frazell (1954) stated that mucoepidermoid carcin ma are composed of three different types of cells (1) epiderm id cells, often fully squamous in character (2) mucus-secreting large cylindrical cells and (3) poorly differentiated cells, denoted a intermediate cells. In most tumours, all three cell types are mixed in varying proportions. Both Stewa t Foote & Becker and Foote & Frazell described the histological characteri tics of a low and high-grade malignant type of tumour the latter with more immature cell structure

In the present series, the microscopical picture of highly differentiated tumour f the low grade type was found in 4 cases. All three types of cells were present with a pred minance ither of squamous, epidermoid cell or mucus-containing cells, frequently forming cystl paces. In the remaining 2 cases, th microscopical features were those of a poorly differentiated tumour with les numerou squam us and mucus-secreting cells, nd a pred minance f intermediate nd poorly differenti ted epidermoid cells. Thi tumou tructure corresponds to the high-grade malignant type described by Stewart, Foot & Becker among others.

Cl nical d t

Fi of the 6 patient wer men. The age t operation ranged from 41–72 years (mean 40.3) The interval between onset of th sympt ms and opera-

tion was less than 1 year in 4 cases 2 years in 1 and more than 5 years in the remaining case One patient complained of pain in the tumour region (a high grade malignant tumour)

Follow-up study

All 4 patients with a low grade malignant tumour were operated on before 1961 (1932–1960) and could therefore be followed up for at least 5 years Two of these patients were followed up for 5 years, one patient for 10 and one for 33 years. No metastases were demonstrable in any of these patients but a local recurrence occurred in 2 cases, in both within one year of operation All 4 patients were alive without signs of the tumour disease at the last follow up examination.

The 2 patients with a high grade malignant type of mucoepidermoid carcinoma were operated on in 1961 and 1963 respectively At operation, both had metastases to regional cervical lymph nodes. One patient was alive without signs of the tumour disease 4 years after operation The other died of the tumour disease 2 years after operation

Treatment

Five of the 6 patients were treated by a combination of surgery and radiotherapy and one by surgery alone Surgical treatment consisted of excision of the tumour in the 4 patients with a low grade malignant tumour In the 2 with a high grade malignant tumour evacuation of the submandibular region was performed combined with radical neck dissection Irradiation therapy was given preoperatively in one case with a low grade malignant tumour postoperatively in another 2 with the same type of tumour as well as in the 2 cases with a high grade malignant tumour No patient was given irradiation both pre- and postoperatively

Malignant Mixed Tumour

Carcinoma in Pleomorphic Adenoma

On reclassification after histological re-examination of the 187 submandibular tumours, 98 were denoted as mixed tumours—95 as benign and 3 as malignant

Histological features

The histological features of the mixed tumour group have been thoroughly described in recent years (Foote & Frazell 1954 Eneroth 1964) and the tumour group has become distinctly delimited owing to a clear histological definition The uncertainty which exists regarding the malignancy depends on the fact that certain histological features (e.g high cellularity cylindromatous structures, incomplete encapsulation) have been

considered to indicate potential malignancy (semi-malignancy) Systematic studies of the correlation between these criteria of "semi-malignancy" and the clinical course in large series (Eneroth 1964 1965 1966) have never theless failed to justify the classification of such tumours as semi malig nant. Thus, a mixed tumour has proved to have a malignant course only when invasive destructive growth is present, as in three cases in this series.

True malignant mixed tumours—i.e pleomorphic tumours containing several components and concurrently incontrovertible criteria of malignancy—are rare In most cases, one of the tumour s components develops into malignant process. Consequently *carcinoma in pleomorphic adenoma* would be a more adequate term for a tumour of this kind.

In all 3 cases which, in the present series, were classified as mixed tumours in view of pleomorphic components in the primary tumour only one component exhibited malignant features In one case, this had the nature f a mesenchymal sarcoma, most closely resembling a fibrosarcoma. In nother case the tumour contained a carcinomatous component best corresponding to a high-grade malignant mucoepidermoid carcinoma (Fig. 4) The lymph node metastases in this case showed only the cancer components from the primary mixed tumour In the third case a solid undifferentiated carcinoma was present within a pleomorphic tumour tissue

Clinical data

Two of the 3 patients with a malignant mixed tumour were men. The age at operati n was 56, 61 and 71 years (mean 63.3) The interval between onset of the symptoms and operation was less than 1 year in one case nd more than 5 years in the other 2 None of the patients had a hi tory of pain.

Follow-up study

Two of the patients were operated on before 1961 (1926, 1960) and could therefore be followed up for at lea t 5 years.

One of these patients, whose tumour had a fibrosarcomatous appearance, died f the tumour disease a few months after operation He had metastases t the region l lymph nodes, th lungs and skel ton The other patient died 2 years after operation, but with no igns of the tumour disease

Treatm nt

All 3 pati nt were treated by a combinati n of surgery and radiotherapy Surgical treatment consisted of evacuation of the submandibular egion nd, i 2 of the 3 cases, of radical neck dissection a well Irradia tion therapy wa given in 2 cases both pre and postoperatively and in one preoperati ly only

tion was less than 1 year in 4 cases 2 years in 1 and more than 5 years in the remaining case One patient complained of pain in the tumour region (a high grade malignant tumour)

Follow-up study

All 4 patients with a low-grade malignant tumour were operated on before 1961 (1932–1960) and could therefore be followed up for at least 5 years Two of these patients were followed up for 5 years, one patient for 10 and one for 33 years No metastases were demonstrable in any of these patients, but a local recurrence occurred in 2 cases, in both within one year of operation All 4 patients were alive without signs of the tumour disease at the last follow up examination.

The 2 patients with a high grade malignant type of mucoepidermoid carcinoma were operated on in 1961 and 1963 respectively At operation both had metastases to regional cervical lymph nodes One patient was alive without signs of the tumour disease 4 years after operation The other died of the tumour disease 2 years after operation

Treatment

Five of the 6 patients were treated by a combination of surgery and radiotherapy and one by surgery alone Surgical treatment consisted of excision of the tumour in the 4 patients with a low grade malignant tumour In the 2 with a high grade malignant tumour evacuation of the submandibular region was performed combined with radical neck dissection Irradiation therapy was given preoperatively in one case with a low grade malignant tumour postoperatively in another 2 with the same type of tumour as well as in the 2 cases with a high grade malignant tumour No patient was given irradiation both pre- and postoperatively

Malignant Mixed Tumour

Carcinoma in Pleomorphic Adenoma"

On reclassification after histological re-examination of the 187 submandibular tumours, 98 were denoted as mixed tumours— 95 as benign and 3 as malignant

Histological features

The histological features of the mixed tumour group have been thoroughly described in recent years (Foote & Frazell 1954 Eneroth 1964) and the tumour group has become distinctly delimited owing to a clear histological definition The uncertainty which exists regarding the malignancy depends on the fact that certain histological features (e g high cellularity cylindromatous structures incomplete encapsulation) have been

Epidermoid Carcinoma

Altogether 11 of the 62 malignant submandibular tumours in the present series showed the well-known histological features of epidermoid carcinoma. No mucus or intermediate cells were demonstrable in the tumours, which showed keratinization of varying degree. Although all tumours were located within the submandibular region, the possibility of metastasis from an epidermoid carcinoma originating elsewhere was considered. In no case, however, was any other primary tumour detected at repeated thorough clinical examination during the follow up period. All 11 tumours must therefore be regarded as primary in the submandibular gland.

Clinical data

Of the 11 patients with epidermoid carcinoma 8 were men and 3 were women. The age at operation ranged from 50–90 years (mean 64.7). The interval between onset of symptoms of the primary tumour and operation was less than 1 year in 8 cases, and more than 5 years in only one case. A history of pain was present in 3 patients.

Follow-up study

In 10 of the 11 patients, operation was performed before 1961 (1924–1960) and they could therefore be followed up for at least 5 years.

Metastases to regional cervical lymph nodes were demonstrable in 3 patients. In all 3 cases, the interval between detection of the primary tumour and observation of metastasis was less than 1 year. Local recurrences occurred in 5 of the 10 cases, in all of them within 1 year of operation.

Five of the 10 patients died of the tumour disease, 3 of them 1 year after operation, and the other two 1 / and 2 years respectively after it. One patient died without signs of the tumour disease 1 year after operation. Table 4 shows the determinate survival rate after 5, 10 and 15 years observation.

It is evident from Table 4 that 10 patients were followed for at least 5 years, and 6 for at least 10 and 15 years. The 2 surviving patients in the group observed for at least 15 years were completely asymptomatic at examination 18 years after operation. It can also be inferred from the table that it seems possible to predict the prognosis as soon as after 5 years follow up, since the determinate survival rate was about the same after 10 and 15 years observation as after 5 (40 and 44.4 per cent respectively).

Treatment

All 11 patients were treated by a combination of surgery and radiotherapy. Surgical treatment consisted of excision of the tumour in 3 cases, and evacuation of the submandibular region in 8. In 2 cases, evacuation of



FIG. 4. Malignant mixed tumour. (a) Pleomorphic structure. Photomicrograph, ×60. (b) Same tumour. Malignant component resembling high-grade malignant mucoepidermoid carcinoma. Photomicrograph, ×650.

TABLE 5 *Solid undifferentiated carcinoma of the submandibular gland 5–15 year follow-up study*

Observation period (years)	No of patients	Died		Determinate survival rate		
		Of tumour disease	Without signs of tumour disease	No of patients	No. of survivals	Survival %
5	10	8	1	9	1	11 1
10	6	5	1	5	0	0

of the 5 cases, the metastases were detected within 1 year of operation and in the remaining case not until > 5 years after it Local recurrences occurred in 5 of the 10 patients within 1 year of operation. Altogether 8 of the 10 patients died of the tumour disease 6 within 1 year of operation, and the other two 2 and 3 years, respectively after it. No correlation could be found between tumour structure and survival time Two patients died without signs of the tumour disease 1 and 8 years, respectively after operation. Table 5 shows the determinate survival rate after 5 and 10 years observation

It is evident from Table 5 that 10 patients were followed for at least 5 years, and 6 for at least 10 Only 1 patient survived for more than 5 years after operation, and none was alive 10 years after it The only surviving patient in the 5-year group died 8 years after operation, without signs of the tumour disease

The determinate survival rate is extremely low i.e 11 per cent after years observation, and 0 per cent after 10 years

Treatment

In 14 of the 15 cases, treatment consisted of a combination of surgery and radiotherapy The remaining patient was operated on without pre- or postoperative irradiation. Surgical treatment consisted of excision of the tumour in 8 cases, and evacuation of the submandibular region in 7 In 2 cases, the latter procedure was combined with radical neck dissection

Irradiation therapy was given both pre and postoperatively in 4 cases, preoperatively only in none and postoperatively only in 10 cases.

Miscellaneous Types of Malignant Tumours

Histological re-examination of the whole material disclosed one acinic cell carcinoma, whose typical histological features were thoroughly described by Eneroth, Jakobsson & Blanck (1966) In addition, we found one patient with malignant tumour classified as a malignant lymphoma, most probably a lymphosarcoma.

TABLE 4 *Epidermoid carcinoma of the submandibular gland 5–15 year follow-up study*

Observation period (years)	No of patients	Died: Of tumour disease	Died: Without signs of tumour disease	Determinate survival rate: No of patients	No. of survivals	Survival %
5	10	5	1	9	4	44.0
10	6	3	1	5	2	40 0
15	6	3	1	5	2	40 0

the submandibular region was combined with radical neck dissection Irradiation therapy was given both pre and postoperatively in 8 cases, preoperatively only in 2 and postoperatively only in 1

Solid Undifferentiated Carcinoma

At histological re-examination of the present material the tumours from 15 patients showed the histological features of a solid undifferentiated carcinoma The tumours were either composed of solid cords of immature undifferentiated malignant cells of varying size and shape (7 cases) or were entirely diffuse, with a sarcomatous like structure (8 cases) In 6 cases the tumours were composed of small somewhat elongated cells (Fig 5) and in 9 cases the tumours were dominated by large, anaplastic cell types (Fig 6) The cellular types could not, however be accurately identified, in view of their undifferentiated nature In the absence of any other demonstrable primary tumour all these tumours could be regarded as primary in the submandibular gland

Clinical data

In this group 8 patients were men and 7 were women. The age at operation ranged from 29–70 years (mean 54 1) The interval between onset of symptoms of the primary tumour and operation was less than 1 year in 11 cases, and more than 5 years in 3 of the remaining 4 There was a history of pain in only one case

Follow-up study

In 10 of the 15 patients operation was performed before 1961 (1926–1960) and they could therefore be followed up for at least 5 years. Metastases were demonstrable in 5 of these 10 patients In 4 cases, the metastases were to regional cervical lymph nodes 3 of these patients had in addition metastases to the lungs Metastases to the lungs without demonstrable regional lymph node metastases developed in another patient In 4

The patient with an acinic cell carcinoma was a man aged 40 years at operation, which consisted of evacuation of the submandibular region. He was alive and without signs of the tumour disease 8 years later.

The patient with a malignant lymphoma was a woman aged 61 at operation. She died of the tumour disease within a year and had distant metastases.

DISCUSSION

Reclassification of totally 187 tumours originating in the submandibular gland, registered at Radiumhemmet from 1909 to 1965 and mostly operated on at the Department of Otolaryngology, Karolinska Sjukhuset, disclosed 125 to be benign or allied non-neoplastic conditions, and 62 to be malignant. The histological criteria used for definition of the benign and malignant types of tumour were in conformity with those earlier applied by one of us (Eneroth, 1964). In most types, the histological structure was so incontrovertible that diagnosis presented no difficulties. Differential diagnostic problems were, in fact, encountered only in the groups of malignant mixed tumours and undifferentiated carcinoma.

Histologically the tumour types registered did not differ from those observed in tumours of the parotid gland (e.g. Eneroth, 1964). Differences from tumours of the latter gland were, on the other hand, noted with respect to the relative distribution of the various types. Moreover, the much lower incidence of tumours of the submandibular gland than of the parotid is noteworthy.

Incidence

We were able to compare two series of tumours—of the parotid and the submandibular gland, respectively—that were equivalent as regards the time and place of treatment. The former series consisted of 1678 true neoplasms of the parotid gland registered at Radiumhemmet from 1909 to 1958, inclusively (Eneroth, Blanck & Jakobsson, unpublished data). During this period 113 true neoplasms of the submandibular gland were also registered at Radiumhemmet, and are included in the present series.

It was apparent that the submandibular tumours amounted to only 6.3 per cent of the whole material, as compared to 93.7 for the parotid tumours. This incidence is in good agreement with the finding of Simons, Beahrs & Woolner (1964) that about 8 per cent of the major salivary-gland tumours involved the submandibular gland. According to Durrant (1964) this large difference between the incidence in the two glands suggests that there must be some factors at work that make the parotid susceptible to tumours or, perhaps, others that render the submandibular gland relatively tumour resistant. The difference could not be based on size alone, because the two parotids weigh 40–60 g, while the two submandibular glands weigh 20–30 g (Seifert, 1966).

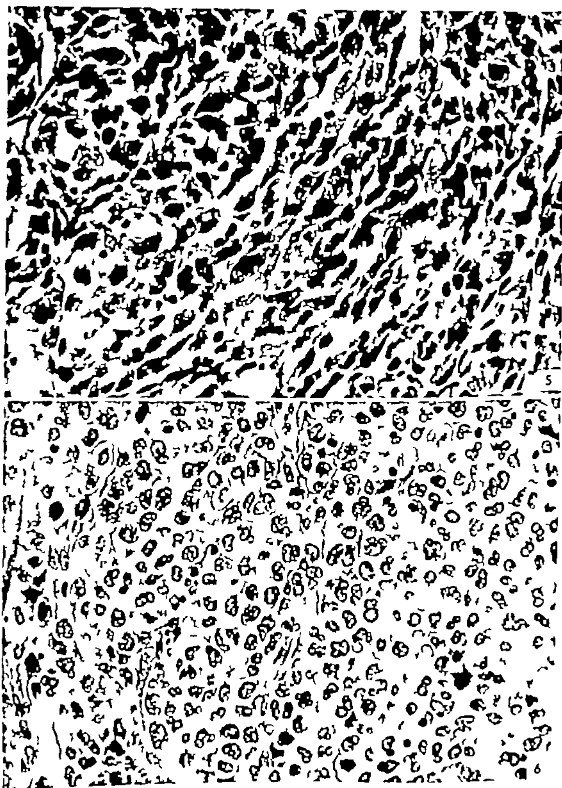


Fig. 5 Solid, undiffere ti ted carc ma Diff se with mall som what l ng ted cell types. Photomicrograph, 400

Fig. 6 Solid undifferentiated carcin m Diffuse with l rg napl ti cell types. Photomicrograph, 400

The interval between onset of the symptoms and operation was considerably shorter in the presence of a malignant tumour than of a benign one in which the preoperative duration was more than 5 years in over 50 per cent of the material (Eneroth & Hjertman, 1966) This applied in only 16 of the 62 patients with a malignant submandibular tumour and 9 of them had an adenoid cystic carcinoma, which is known for its slow growth but nevertheless exceedingly poor long-term prognosis (Eneroth, 1966 Blanck, Eneroth & Jakobsson 1967) In 29 cases—i.e. about half of the malignant submandibular tumours—the preoperative duration was less than 1 year

The present investigation shows the difficulty of making a differential diagnosis between benign and malignant submandibular tumours on the basis of the symptomatology even if there is a higher incidence of short duration and of pain in the latter In view of the high incidence of absence of pain and the not infrequently long duration of malignant tumours, a painless tumour of many years duration is not a guarantee of its lack of malignancy

Histological Features and Clinical Course

Adenoid cystic carcinoma

In all 25 cases the structures were typical of this type of tumour The well known structural components, cribriform and solid, were present in varying proportions in the individual tumours A study was made of the correlation between structural type and mortality in the tumour disease (Table 2) and of the respective survival time (Fig 4) It showed that tumours with a predominance of solid structures had a much more malignant course with significantly higher mortality and shorter survival time than those in which cribriform structures predominated Even though the number of cases with a fatal outcome amounted to only 12 we considered it justified to regard the predominantly solid types of adenoid cystic carcinoma as more poorly differentiated and more malignant than the others. Consequently we feel that it is warranted to classify adenoid cystic carcinoma into low-grade and high-grade malignant types on the basis of the histological structure in conformity with the corresponding classification of mucoepidermoid carcinoma (e.g. Linell 1948 Beahrs *et al* 1960)

A possible explanation of the poorer prognosis in the predominantly solid tumours is that they could be regarded as solid undifferentiated carcinomas, a type of tumour with a recognized high grade of malignancy and poor prognosis However in all 4 cases of predominantly solid tumours classified as adenoid cystic carcinoma a distinct cribriform component was observed. Among the tumours denoted as undifferentiated carcinoma 3 were observed with a similar cell structure but no cribriform structures were present that could have motivated the diagnosis of adenoid cystic carcinoma

All tumours exhibited infiltrative growth, and no correlation was demon

As far as the relative distribution of the tumour types in the parotid and the submandibular gland is concerned many authors state that a higher percentage of tumours of the latter gland are malignant than those of the former gland (e.g Frazell 1954 Simons Beahrs & Woolner 1964) This conclusion is in accordance with our observations, i e., that 299 of 1678 parotid tumours (17 8 per cent) were malignant (Eneroth Blanck & Jakobsson unpublished data) as compared to 42 of 113 submandibular tumours (37.2 per cent) registered at Radiumhemmet from 1909 to 1958. This implies that malignant tumours are more than twice as common in the submandibular gland as in the parotid The incidence of adenoid cystic carcinoma, solid undifferentiated carcinoma and epidermoid carcinoma in the present series of submandibular tumours (about 13 8 and 6 per cent respectively) is considerably higher than that of the same types of tumour (about 2, 1 and 0 1 per cent respectively) in the parotid (Eneroth 1964) The different histology of the two glands is a possible explanation In addition to differences in the duct system there is a difference in the acini those of the parotid gland being entirely composed of serous cells, whereas the acini of the submandibular gland contain mucus cells as well as serous cells

Sex and Age

As far as the sex incidence in the whole material (187 cases) is concerned the female patients predominate over the males If on the other hand the series is divided into benign and malignant neoplasms, a distinct sex difference appears in the former group as compared to the latter Thus, only about 1/3 of all the patients with a benign submandibular tumour were men whereas they comprised 36 of the 62 patients (about 60 per cent) of those with a malignant tumour

The average age at operation of patients with a malignant submandibular tumour was 56.5 years, i e., about 10 years more than that of patients with a benign tumour at this site (Eneroth & Hjertman 1966) Simons, Beahrs & Woolner found in their series that the average age of patients with a malignant tumour was about 8 years higher than that of patients with a benign tumour

Symptoms and Signs

Apart from a palpable swelling in the submandibular region symptoms and signs were rare There was, however a history of pain in a slightly higher incidence of the malignant tumours (12 of 62 cases, i e., about 19 per cent) than of the benign ones in which only about 3 per cent of the patients complained of pain (Eneroth & Hjertman 1966) Adenoid cystic carcinoma and epidermoid carcinoma in particular were responsible for a relatively high incidence of pain (in almost 30 per cent) and especially the former is known for its tendency to peri and intraneural growth (Eneroth 1966 Blanck Eneroth & Jakobsson 1967 among others)

patients with a low-grade malignant tumour (well-differentiated type without invasive growth)

Malignant mixed tumour

The difficulties involved in the histological diagnosis of a malignant mixed tumour have frequently been pointed out (e.g. Eneroth, 1964). The existence of a fairly large amount of fibrous stroma with a high collagen content within a malignant salivary-gland tumour may be said to represent a pleomorphic component. However, in our experience—based on more than 1800 salivary-gland tumours—such a stroma is, in general, a highly subjective and inaccurate sign of a pleomorphic tumour. This applies especially if irradiation therapy has been given preoperatively. Although some help may be obtained from the type of arrangement of the cellular components, the diagnosis of malignant mixed tumour remains uncertain and in most cases, questionable.

In the present series, 98 tumours were denoted as mixed tumours, 3 of them (3 per cent) as malignant. We are nevertheless well aware that these diagnoses may be subject to doubt. A metastatic lesion was available for histological examination in only one case. One malignant component alone was demonstrable, and resembled a high-grade malignant type of mucoepidermoid carcinoma. It can presumably be questioned whether there are in fact, mixed tumours which, retaining their pleomorphic structure, spread in the organism and exhibit the structure of a mixed tumour in metastases as well (Moberger & Eneroth). No conclusions about the grade of malignancy of the 3 tumours denoted as malignant mixed tumours could be drawn from the follow-up study in the present series.

Epidermoid carcinoma

The major problem involved in the diagnosis of this tumour type was not histological identification of the tumour but rather whether it was actually a metastasis from a carcinoma originating elsewhere or a primary tumour. No other tumour was detected during the follow up study in any of the 11 patients. The possibility must also be taken into account of a primary carcinoma originating from tissue components in the immediately adjacent tissue (e.g. a congenital cyst). Such a possibility nevertheless seems most unlikely.

An epidermoid carcinoma in this region represents a fairly malignant type of tumour with a comparatively short preoperative duration, pain, local recurrences, and metastases to regional cervical lymph nodes in a fairly high incidence. Even after a 5-year follow-up the determinate survival rate is less than 50 per cent but—in contrast to adenoid cystic carcinoma (Table 3)—the rate does not fall further when the observation period is extended to 10 and 15 years, respectively (Table 4).

This implies that in epidermoid carcinoma the prognosis can be evaluated

strable between survival time and different types of such growth (e.g invasion of peripheral nerves or vascular invasion) In the cases with metastases the tumour structure was the same in primary tumour and metastasis Moreover the tumour structure was unchanged in the late local recurrences which took place during a follow up period which, in some cases, exceeded 20 years Thus, in none of the 25 cases of adenoid cystic carcinoma could any change be observed in the tumour structure that was indicative of gradual transition into malignancy A noteworthy feature is however the long time that had elapsed in some cases between primary treatment and the appearance of a local recurrence or metastasis In view of the often slow growth of adenoid cystic carcinoma an extremely long observation period is required to permit a definite prognosis (Simons, Beahrs & Woolner 1964 Eneroth 1966 Blanck, Eneroth & Jakobsson 1967)

Because of the long observation period in the present series, the prognosis *quoad vitam* could be shown to be much poorer in adenoid cystic carcinoma than can be inferred from the follow up studies which generally cover 5 years only A 5 year follow up period does in fact, give a too optimistic view of the prognosis of this type of tumour which is evident from the determinate survival rate after 5 10 and 15 years (Table 3) Although adenoid cystic carcinoma has a grave prognosis when it involves the parotid gland (Blanck Eneroth & Jakobsson 1967) the long term prognosis is definitely poorer in the present series of submandibular tumours The explanation might be the average longer preoperative duration of the tumour i e., about 6 years as compared to 3 years in the parotid series (Eneroth 1966) The preoperative duration may actually have played a role in the incidence of metastases, which was 75 per cent in the present series in comparison to 43 per cent in the series of parotid tumours reported by Blanck, Eneroth & Jakobsson (1967)

Mucoepidermoid carcinoma

In a large series of parotid neoplasms, mucoepidermoid carcinoma comprised about 23 per cent of all malignant tumours (Eneroth 1964) In the present study only 6 of the 62 malignant submandibular tumours (about 10 per cent) could be classified as mucoepidermoid carcinoma All the tumours were easily identifiable through the typical histological structures described by Jakobsson Blanck & Eneroth among others.

The number of cases is too small to contribute to the discussion about the justification of distinguishing a low grade from a high grade malignant type (e.g Linell 1948 Bruzelius *et al* 1957 Beahrs *et al* 1960)

Despite the small number of cases, the follow up study may support the existence of a difference between the malignancy in the two groups. This is because the 2 patients with a tumour denoted as of high grade malignancy had metastases whereas this did not apply to any of the 4

eeivable explanation is the longer preoperative duration of the tumour and the higher incidence of metastasis that may be associated with it in tumours of the former gland. This emphasizes the importance of early diagnosis so that radical surgical measures can be taken at the earliest possible stage

No definite conclusions about the effect of irradiation can be drawn from the present study which is based on an operated series.

ZUSAMMENFASSUNG

Die histologische R klassifizierung von 187 in der Submandibularis primären Tumoren zeigte in 62 Fällen maligne Tumortypen Eine langjährige klinische Beobachtungszeit zeigt dass die malignen Submandibularistumoren — verglichen mit e tsprechenden Tumorformen der Parotis — ein ungünstigere Prognose aufweisen. Ausserdem wurde gez igt, da s denoid zystische Karzin me in niedrig- und hochgradig maligne Typen eingeteilt werden können. Bei schnell wachsenden Tumoren wie epidermoidem Karzin me, solidem undifferenziertem Karzinoma und hochgradig malignem Typ von aden id-zystischem Karzinom zeigt sich die ungünstige Prognose bereit bei einer 5jährigen Beobachtungszeit. Aus der orliegenden Arbeit geht hervor dass für eine d finitive Prognose bei dem langsamer wachsenden niedrig malignen Typ von denoid-zystischem Kar zinom eine viel längere Beobachtungszeit notwendig ist. In Anbetracht des hohen Vorkommens von malignen Tumoren der Submandibularis besteht grosse Gefahr dass eine Veränderu g in dieser Regi n tatsächlich malign sein kann Di Bedeutung iner möglichst frühen rad kalen chirurgischen Behandlung wird hervorgehoben.

REFERENCES

Ahlbom, H E., 1935 Mucous- nd salivary-gland t mours. A ! *Radiol* (Stockh.) Suppl. 23.

Bea rs, O H Woolner, L. B., Ca rter, S. W nd Devine, K. D., 1960 Surgical management f parotid lesions. *Arch S rg Chic)* 80 890

Beatys, W E., 1963 Primary mi ed t mors of the submaxillary gland. *Med. Ann. D.C.*, 32, 53.

Blanck, C., E kroth, C.-M and Jakobsson, P A., 1967 Adenoid cystic carcinoma of the parotid gland T be published in *Act Rad ol* (Stockh.)

Bauman n, K., Croneqvist E., Linell, F nd Bene , F 1957 Tumours of the parotid gland. A histological study and res lts of combined rgical nd radiological treatment. *Act Ch Scand* 111 1

Dockerty M. B., nd M yo, C W 1942 Primary tumors f the submaxill ry gland ith pecial reference t mi ed t mors. *Surg Gynec Obst t* 74 1033.

De L K. M., 1961 Mal gn nt mixed t mors f the submaxillary sali ry gland. *Plast Reconst Surg* 33 237

Eneroth, C. M 1964 Histological nd cli ical aspect of parotid t mours. *Act Oto-laryng* (Stockh. S ppl 191

1965 Mixed tumors f major sali ry gland Prognostic rol of capsula structure *A n. Otol* 3 944

— 1966 Zur Prognose des denoid-zystischen Carcinoms der grossen Speicheldrüsen. *Arch. Oh Nas. K hlk pfheilk* 188 309

F ekers, C. M., nd Hjertm n, L., 1966 Mi ed t mours of the submand bula gland. *J Laryng* 80 230.

—contrary to that in adenoid cystic carcinoma—on the basis of 5-year follow up The quicker manner of growth and metastasis of epidermoid carcinoma result in more rapid death than in cases of well-differentiated adenoid cystic carcinoma (low grade malignant type) Patients surviving the first 5 year period have a favourable later prognosis In adenoid cystic carcinoma on the contrary the long term prognosis is gloomy—i.e., no survivals after 15 years

Solid undifferentiated carcinoma

The somewhat high degree of variability of the histological structures in the tumours included in this group gives evidence that they form a heterogeneous group The variation applies both to the cellular structure (varying cell size and degree of cellular polymorphism) and to the arrangement of the malignant cells (solid cords or entirely diffuse) We could not however demonstrate any correlation between the structural details and the prognosis.

The consistently short duration of symptoms and the high incidence of local recurrence and metastasis are indicative of high-grade malignancy The gloomy prognosis in this type of tumour is, however most apparent from the determinate survival rate which falls from 11 to 0 per cent after 5 and 10 years follow up respectively

Miscellaneous types of malignant tumours

The fact that we found an acinic cell carcinoma among the submandibular tumours confirms the existence of such tumours in this region No diagnostic problem was involved and the tumour structure was entirely in conformity with previous descriptions (e g Eneroth Jakobsson & Blanck, 1966) The other tumour in this group was a lymphosarcoma It is open to question whether the tumour was primary in the submandibular gland

Treatment

In the present series, surgical treatment consisted in 40 cases of evacuation of the submandibular region combined in 15 of the 40 with radical neck dissection In the other 22 cases the tumour was excised without concurrent neck dissection Surgical treatment was thus considerably more radical in the case of submandibular tumours than in that of parotid tumours during the same period (Eneroth Jakobsson & Blanck 1966 Blanck Eneroth & Jakobsson 1967)

In view of the high incidence of metastasis to cervical lymph nodes, radical neck dissection is mandatory in the presence of a malignant tumour of the submandibular gland

In the same type of tumour the prognosis was found to be poorer with localization to the submandibular gland than to the parotid gland A con

ceivable explanation is the longer preoperative duration of the tumour and the higher incidence of metastasis that may be associated with it in tumours of the former gland. This emphasizes the importance of early diagnosis, so that radical surgical measures can be taken at the earliest possible stage.

No definite conclusions about the effect of irradiation can be drawn from the present study which is based on an operated series.

ZUSAMMENFASSUNG

Die histologische Reklassifizierung von 187 in der Submandibularis primären Tumoren zeigte in 62 Fällen maligne Tumortypen. Eine langjährige klinische Beobachtungszeit zeigte, dass di malignen Submandibularistumoren — verglichen mit entsprechenden Tumorformen der Parotis — eine ungünstigere Prognose aufweise. Ausserdem wurde gezeigt, dass adenoid- ystisch Karzinom in niedrig- und hochgradig maligne Typen eingeteilt werden können. Bei schn ll wachsenden Tumoren wie epidermoidem Karzi ma, solidem undiffere ziert m Karzinoma und hochgradig malignem Typ von adenoid-cystischem Karzinom zeigt sich die ungünstige Prognose bereit bei iner 5jährigen Beobachtungszeit. Aus der vorliegenden Arbeit geht hervor, dass für ein definitive Prognose bei dem langsamer wachsend n niedrig malignen Typ von adenoid-zystischem Kar zinom ine viel längere Beobachtungszeit notw ndig ist. In Anbetracht des hohen Vorkomme s on malignen Tumoren der Submandibularis best ht grosse Gefahr, dass eine Veränderung in d eser Region t tsächli h malign sein ka n. Die Bedeutung iner möglichst frühen radikalen chirurgischen Behandlung wird hervorgehoben.

REFERENCES

AHLBOM, H. E., 1935. Mucous- nd salivary-gland t mours. *A t Radiol.* (Stockh.) S ppl. 23.

BEAHRS, O. H., WOOLNER, L. B., CA VERT, S. W. and DEVINE, K. D. 1960. Surgical management of parot d lesions. *Arch. Surg.* (Chic. *80*, 890.

BEA RS, W. E., 1962. Primary mixed tumors f the submaxillary gland. *M d. Ann. D.C.*, 32, 46.

BLANCK, C., ENEROTH, C. M., nd J KOBSSON, P. A., 1967. Adenoid cystic carcinoma f the parotid gland. T be published in *Act Rad ol.* (Stockh.)

BAUMGARTEN, S. CRONQVIST, E., LIVELL, F. nd BERMAN, F. 1957. Tumours of the parotid gland. A histological study and result f combined surgical nd radiological treatment. *Act Chir. Scand.* *113*, 1.

DOCKERTY, M. B., nd M YO, C. W. 1942. Primary t m rs of the submaxillary gland with special reference t mixed t mors. *S rg. Gynec. Obst.* *t 74*, 1033.

DUNMA, K. M., 1964. Malignant mixed t mors of the submaxillary sal ry gl d. *Plast. Reconst. Surg.* *33*, 237.

ENEROTH, C. M., 1964. Histological nd clinical pect f parotid t mo rs. *Act Oto-laryng.* (Stockh.) Suppl. *191*.

— 1965. Mixed tumors of major sali ry gland. Prognostic role of capsular structure. *Ann. Otol.* *74*, 944.

— 1966. Zur Prognose des adenoid-zystischen Carcinoms der grossen Speicheldrüsen. *Arch. Ohr. Nas. K hlkopfheilk.* *186*, 309.

ENEROTH, C.-M., and HAMBERGER, L., 1966. Mixed tumours f the submandibula gl nd. *J. Laryng.*, *80*, 830.

—contrary to that in adenoid cystic carcinoma—on the basis of 5-year follow up The quicker manner of growth and metastasis of epidermoid carcinoma result in more rapid death than in cases of well-differentiated adenoid cystic carcinoma (low grade malignant type) Patients surviving the first 5-year period have a favourable later prognosis. In adenoid cystic carcinoma on the contrary the long term prognosis is gloomy—i.e no survivals after 15 years

Solid undifferentiated carcinoma

The somewhat high degree of variability of the histological structures in the tumours included in this group gives evidence that they form a heterogeneous group The variation applies both to the cellular structure (varying cell size and degree of cellular polymorphism) and to the arrangement of the malignant cells (solid cords or entirely diffuse) We could not, however demonstrate any correlation between the structural details and the prognosis.

The consistently short duration of symptoms and the high incidence of local recurrence and metastasis are indicative of high-grade malignancy The gloomy prognosis in this type of tumour is, however most apparent from the determinate survival rate which falls from 11 to 0 per cent after 5 and 10 years follow up respectively

Miscellaneous types of malignant tumours

The fact that we found an acinic cell carcinoma among the submandibular tumours confirms the existence of such tumours in this region No diagnostic problem was involved, and the tumour structure was entirely in conformity with previous descriptions (e.g Eneroth Jakobsson & Blanck, 1966) The other tumour in this group was a lymphosarcoma It is open to question whether the tumour was primary in the submandibular gland

Treatment

In the present series, surgical treatment consisted in 40 cases of evacuation of the submandibular region combined in 15 of the 40 with radical neck dissection In the other 22 cases, the tumour was excised without concurrent neck dissection Surgical treatment was thus considerably more radical in the case of submandibular tumours than in that of parotid tumours during the same period (Eneroth Jakobsson & Blanck 1966 Blanck Eneroth & Jakobsson 1967)

In view of the high incidence of metastasis to cervical lymph nodes, radical neck dissection is mandatory in the presence of a malignant tumour of the submandibular gland

In the same type of tumour the prognosis was found to be poorer with localization to the submandibular gland than to the parotid gland A con

Acta oto-laryngologica 64 437-442 (1967)

A STUDY OF EXPERIMENTAL DEAFNESS IN HUMAN VOLUNTEERS WITH A NEW ORAL ANTIDIABETIC DRUG R 94

J N De Sa and K B Bhargava
Bombay India

From the E.N.T Department K.E.M Hospital Pa el Bombay 12

) R 94 (N Benzin Sulphonyl-N Isopropyl Urea) a new synthetic Anti Diabetic drug when used in the treatment f Diabetis caused a reversibl deafness affecting th higher frequencies. b) Thi deafness increases with the continuatio f the intak of the drug, and is relieved on removal of the drug o th administration of a vasodilat r drug, e g Nicotini A id in a daily dosage of 200 mg. c) A study of the drug R 94 on 26 human olunteers i presented.

Various therapeutic agents used in the treatment of medical conditions have been known to cause damage to the human labyrinth. The use of such ototoxic drugs as streptomycin, dehydrostreptomycin, neomycin, kenamycin, quinine salicylates and analyn dyes have resulted in irreversible damage to the human ear The site of action of these drugs are on the vestibular or cochlear counterpart of the human ear

R. 94 (N Benzine Sulphonyl N Isopropyl Urea) a new synthetic anti diabetic drug, closely related to tolbutamide and found to be five times more effective in animal experiments (Tonse *et al.*, 1962) was developed at the Haffkine Institute, Parel Bombay 12.

The clinical trial of this drug on patients suffering from diabetes at the Diabetic Centre of the K.E.M Hospital, Parel Bombay 12 proved its effec tiveness as an hypoglycaemic agent, but also revealed an important side-effect, namely sudden deafness, which appeared within 4 hours of ad ministration and was associated with tinnitus in a few cases but there was no vertigo *The deafness was greater with the increase in the dosage of* R 94, but, unlik ther ototoxic drugs, showed a complete regression of the deafness n removal of the drug. This side-effect of the drug on human hearing was thus totally reversible. The fact that the drug has no other known to icity and does not produce hypoglycaemia in normal patients, prompted us to use R. 94 as an experimental drug in the production of deafness in human beings, where the audiometric study can be done much more easily accurately economically and reliably as compared with animal (Schuknecht, 1959)

Knowing the site of action of most of the ototoxic drugs as used today the probable ite f action of this drug, and also the possible antidote was

ENEROTH, C. M JAKOBSSON P A and BLANCK, C., 1966 Acinic cell carcinoma of the parotid gland. *Cancer 19* 1761

FOOTE, F W JR and FRAZELL, E. L., 1954: Tumors of the major salivary glands. *Atla f Tumor Pathology* Sect IV Fasc. 11 Armed Forces Institute f Pathology Washington, D C.

FRAZELL, E. L., 1954 Clinical a pects of tumors of the m jor salivary glands. *Cancer 7* 637

J KOBSSON, P A., BLANCK, C., and ENEROTH C. M Mucoepiderm id carcinoma f the parotid gland. To be published in *Cancer*

LINELL, F 1948: Mucus-secreting nd cy tic epidermoid carcinoma f the mucous- and salivary glands. *Acta Path Microbiol Scand 25* 801

MOBERGER, G and ENEROTH, C. M : Malign nt mi ed tumors of the majo salivary glands. *Cancer* under public tion.

QUATTLEBAUM, F W DOCKERTY M B and MAYO, C. W 1946 Adenoc rcinoma cyll droma type, f th parotid gland. *Surg Gynec Obstet 82* 342.

SEIFERT G 1966 *Doerr Uehlinger Spezielle pathologische Anat mie* Vol 1 Springer V rlag, Be lin, H idelberg, New York.

SIMONS, J N BEAHRS, O H a d WOOLNER, L. B 1964: Tum rs f the submaxillary gland *Am J Surg 108* 485

STEWART F W FOOTE, F W and BECKER, W F 1945 Muco-epidermoid tumors of salivary glands. *Ann Surg 122* 820

THACKRAY A C., a d LUCAS, R B 1960 The hist logy of cylindroma of mucous gland o igin *Brit J Can er 14* 612

C M En roth Dept f Ot l ryngology
Karolinska Sjukhu et Stockholm 60 Sweden

Received May 2 1967

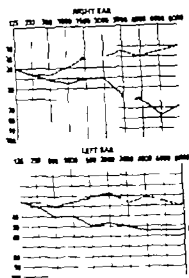


Fig. 3.

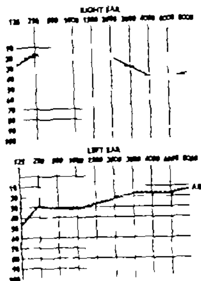


Fig. 4.

Th youngest patient was 15 years and the oldest wa 55 years. All the cases were male patients.

The dosag of R 94 in all these cases was 1 g orally daily either in fractional doses or in one dose The longest period of treatment was 28 d ys. Audi metric tests were done on these patients 4 hours after the daily dosage from th day of admission to hospital Tests were also done when the patients first complained of sul jective deafness, which was inquired of them every day until subsequent recovery

It wa noticed that after a dosage of 3 g, all patients complained of subjecti e deafness and in only 2 cases was the deafness associated with slight tinnitus, while in no case wa there any vertigo Examination of the central nervous ystem revealed n abnormal findings. Cold caloric tests were normal in all cases. Audiograms, taken in all cases within 4 hours of the onset of deafness, showed a decrease in the air conduction threshold f 5 dB t 20 dB, mostly in 3000 4000 and 8000 frequencies (Fig. 1) The bone cond ction threshold also howed a corresponding drop of 5 to 15 dB in th higher frequencies

In 9 of these 28 cases the dosage of 1 g of R 94 was continued in pite f th onset of deafness, f r 7 days With such a prolonged treatment the deafnes wa found t increase from the 3rd day onwards, and was again seen to be marked in the higher frequencies, now with a slight decline in the lower frequencies f 10 to 15 dB (Fig. 2)

In 5 f these cases a los of 50 to 65 dB was n ticed in the higher frequencies, causing great al rm in the patients (Fig. 3) However it was i teresting to not that with t tal cessation of the drug hearing was retrieved gradually returning t the normal pretreatment level in 14 days.

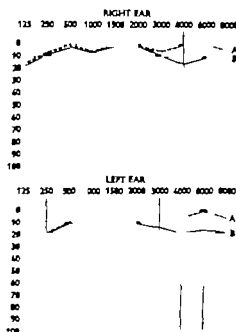


Fig 1

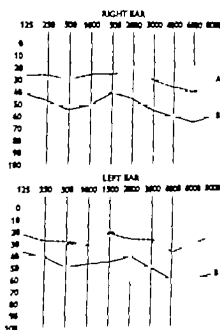


Fig 2

considered From the results of the use of R 04 in the earlier cases it was felt that because of its ototoxic effect appearing within a few hours of its administration and also because of its completely reversible nature its action could be allergic, vasospastic or neural In the light of (1) no clinical symptoms of vertigo (2) occasional tinnitus in a few cases, (3) the absence of recruitment (this could not be done as the deafness was equally bilateral) and (4) the absence of any response with the use of antiallergic drugs the possibility of its action being allergic was ruled out.

Furthermore the spontaneous recovery which occurred on the stoppage of the drug discounted the possibility of its having an action on the neural element—vestibular cochlear or central Such an effect then could only be of a vascular nature which seemed more probable (for even after prolonged administration stoppage of the drug and the employment of a vasodilator drug restored hearing to the pretreatment level) Whether this result was due to the action of the vasodilator drug in the end organ or as a general vasodilatation affecting the centre is not known, but the improvement which resulted was remarkable and confirmatory

Thus armed with the knowledge of the possible site of action and having in hand an effective antidote 26 patients suffering from mild to severe diabetes were selected for this experimental study For the purpose of repeated examinations and audiometric tests these patients were hospitalized in the K E M Hospital Parel Bombay 12 Only cases with normal E.N T findings, no history or typhoid fever or malaria or use of drugs like streptomycin and quinine in the recent past and with normal pure tone audiogram were selected

acid produced after the second dose a subjective relief in the deafness in all 5 cases, but audiometrically it was found that after the administration of nicotinic acid for 3 days, i.e. 300 mg, the audiogram was found to be normal. In this group R 94 was discontinued at the onset of the symptoms of deafness (Fig. 5).

In Group C the dosage of nicotinic acid was 200 mg and here the improvement of the subjective deafness was confirmed within 24 hours by audiogram, showing a complete return of hearing in 3 of the 5 cases where R. 94 was stopped. In two cases the drug was continued in a dosage of 1 g daily+200 mg of nicotinic acid for a period of 14 days, and a daily audiogram taken showed no effect of R. 94 on the hearing, except that both patients experienced slight flushing and palpitation from the nicotinic acid.

This short study describes the side-effects of a new antidiabetic drug R. 94 in producing within 4 hours of its administration what is considered to be ototoxic nerve deafness. This deafness is relieved either by the stoppage of the drug at the onset of the symptoms or by the administration f nicotinic acid. Furthermore if the drug is given for a longer period with an increase in the dosage of nicotinic acid no deafness is produced

This study has given us some very interesting data It has been possible for us to produce experimental deafness in man, a deafness which by irtue of its being caused by a vascular spasm affecting mostly the basal turn of the cochlea, is easily reversed either on st ppage of the drug or by the use of vasodilator medication It would be interesting, however to confirm the site and mode of action of R 94 by animal experimentation, which has n t been possible by the present workers but has been very kindly taken over by Dr Fritz Zöllner and his team at the Hals-Nasen-Ohrenklinik at Freiburg, Germany

ZUSAMMENFASSUNG

E wurd beoba htet, dass P tienten mit Diabetes im Diabetic Ce tre des K.E.M Hospitals, Parel, Bombay 12, Indien, na h Behandl g mit R. 94 (N Benzin Sulpho yl-N-Isopropyl Urea) inem einen synthetischen antidiabetischen Medikament, da sich al wi kungsvolles hypoglykämisches Mitt l erwiese hatte h Nebenersch inung plötzlich a ftretend Taubheit zeigten die innerhalb on 4 Stunden ach Beginn der Beha dlu g einsetzte und sich m t st igender Dosierung verstärkte Im Gegensatz zu anderen bekan ten otot ischen Medikamenten zeigt es si h jedoch, dass na h Entzug des Medikaments eine ollkomme Rückbild g der Taubheit zu normalem Gehör erfolgte woraus ersichtlich ist das diese Nebenerscheinungen von plötzlicher T ubh it vollkommen revers bel sind. Da Fehlen anderer t lscher Erschein ngen bewog uns, die Wirkung dieses Med kament durch die Erzeugung von experimenteller Taubheit n 20 Freiwilligen zu beobachten. Da Alter der Patienten lag zwischen 15 und 16 Jahren. Alle wiesen ein normales Gehör auf und wurden f Grund von wiederh lten diometrischen Bewertungen hospitalisiert und jeweils 4 Stunden nach An

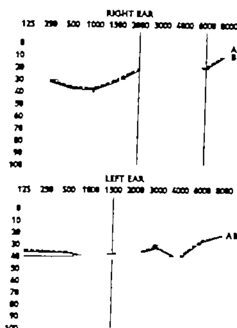


Fig 5

In the remaining 17 cases, 2 cases discontinued from fear of losing their hearing

The remaining 15 were divided into 3 groups

Group A

Consisting of 5 cases where R 94 was given with 100 mg of nicotinic acid by mouth from the 1st day

Group B

Consisting of 5 cases where 100 mg of nicotinic acid was given orally after the onset of deafness on the 3rd day

Group C

Consisting of 5 cases where 200 mg of nicotinic acid was given orally after the onset of deafness.

It was observed that in Group A on reaching 3 g i e the toxic dosage no symptom of deafness was complained of and the audiogram showed no deviation from the pretreatment level (Fig 4) As the dosage of R 94 was continued with 100 m of nicotinic acid daily 4 of the 5 cases revealed a subjective deafness on the 7th day The audiogram taken at the time showed a decline of 5 to 15 dB in the higher frequencies but on increasing the dosage of nicotinic acid to 200 mg the deafness disappeared on the 10th day

In the 5 cases of Group B the administration of 100 mg of nicotinic

Acta oto-laryngologica 64 [illegible] (1967)

HEARING IN MENIERE'S DISEASE

A Study of Pure Tone Audiograms in 334 Patients

A. ENANDER and J. STAHLE

Uppsala, Sweden

From the Department of Otolaryngology (Head: Prof. A. Sjöberg) University Hospital, Uppsala

In 334 patients with Ménière's disease the pure-tone audiograms were analysed, and the age at onset, onset symptoms and bilaterality were studied. The flat curve was most common (60%) followed next by the rising curve (17%) and the falling curve (12%). The average hearing loss for the whole material was 52 dB. Patients with flat, rising and falling curves had hearing loss of approximately the same order of magnitude. Patients with rising and trough curves had on the average the shortest duration of disease. The hearing loss progressed most rapidly during the first year of the disease after which only minor deterioration took place. The mean age for patients with falling curves was on the whole the same as for the whole material.

Fluctuation of the auditory threshold was recorded with all types of curves, but was somewhat more common with rising and trough curves. Fluctuations were recorded in approximately half of the patients, regardless of the duration of the disease and they were noted more often within the frequency range 250–1000 Hz than at higher frequencies. The average size of the recorded fluctuations was 20–30 dB, this being independent, on the whole, of the frequency range.

The disease was bilateral in 14%. In half of these cases the second ear was involved either simultaneously or during the first year of illness. In the other half the second ear was affected after varying intervals of up to 20 years. In 77% of the patients the disease was manifested before the age of 51 years. The most common form of onset of the disease was simultaneous hearing loss and vertigo. Vertigo alone as the onset symptom was noted in 37% of the patients, and hearing loss in 22%. In two-thirds of the latter patients the first vertigo attack occurred after an interval of one year or longer.

INTRODUCTION

In descriptions of the hearing loss in Ménière's disease the configurations of pure-tone audiograms as recommended by Carhart (1945) or variants thereof (Rosberg et al. 1963, among others) have generally been used.

This work was supported by the Swedish Medical Research Council (Project No. 17X-417-02).

wendung des Medikaments untersucht Es zeigte sich dass nach Applikation von 3 g R 94 alle Patienten über subjektive Taubheit klagten in einigen Fällen begleitet von Tinnitus, in keinem Fall jedoch von Vertigo Wir beobachteten eine Kontrollserie von 9 Patienten bei denen kein Antidot angewendet wurde während 7 Tagen Alle diese Fälle zeigten eine zunehmende Taubheit mit markantem Gehörschwellenverlust in den höheren Frequenzen von 4000 und darüber Der Verlust von 50 bis 60 dB wurde notiert Alle diese Fälle waren nach Entzug des Medikaments innerhalb 14 Tagen von ihrer Taubheit befreit

Die übrigen 17 Fälle wurden mit Nikotinsäure behandelt ausgehend von der Annahme dass die Taubheit nachdem sie sich als reversibel erwiesen hatte möglicherweise durch einen vaskulären Spasmus verursacht worden war der die Basalwindung der Cochlea betroffen hatte Diese Fälle wurden in 3 Gruppen eingeteilt a) behandelt mit 100 mg Nikotinsäure täglich beginnend am 1 Tag b) behandelt mit 100 mg Nikotinsäure täglich beginnend am 3 Tag c) behandelt mit 200 mg Nikotinsäure täglich beginnend am 1 Tag der R 94 Behandlung

Bei Anwendung von 100 mg Nikotinsäure vom 1 Tag an trat ein Verlust von 5–15 dB in den höheren Frequenzen ein Dieser Verlust wurde innerhalb von 10 Tagen durch Erhöhung der Nikotinsäuredosen auf 200 mg kompensiert Bei Anwendung von 100 mg Nikotinsäure vom 3 Tag an zeigten die audiometrischen Messungen dass trotz einer subjektiven Besserung der Taubheit das normale Gehör erst durch Erhöhung der Dosis auf 200 mg wiedererlangt wurde Bei Anwendung von 200 mg Nikotinsäure vom 1 Tag an konnte kein Verlust des Gehörs während 14 Tagen festgestellt werden

Zusammenfassend wurde beobachtet dass R. 94 ein antidiabetisches Medikament eine reversible Taubheit mit Wirkung auf die höheren Frequenzen innerhalb von 4 Stunden verursachte Diese Taubheit nahm zu bei Weiterführung der Behandlung und liess nach entweder durch Entzug des Medikaments oder durch Anwendung eines vasodilatorischen Medikaments, wie Nikotinsäure in täglichen Dosen von 200 mg

REFERENCES

SCHUKNECHT E. H 1959 *Dis ses of th Nos Throat and Ear* (C. J ckso and C. L. Ja kson) p 383 W B. Saunders, Phil d lphia

TONER, *et l* 1962 *A tu 891* 193

E N T Departm nt KEM Hospit l
Parel Bombay 12 Indi

Received August 1 1967

temporal development of the hearing loss Since a large and uniformly investigated series had been collected at the Uppsala clinic over a period of a few years, such an analysi was found possible The primary aim of our investigation was to study the configuration of the pure-tone audiogram in relation to the duration of the disease and to the degree of hearing loss. In addition, factors such as age at onset, onset symptoms, bilaterality and fluctuations are considered

MATERIAL AND METHODS

The material consi ted of 334 patients, of whom 160 (48%) were women and 174 (52%) men, and wa collected consecutively over a period of just over 5 years. To a certain extent the material was selected, in that it in cluded 210 patients who had such severe vertigo that they were treated surgically with ultrasonic irradiation. The remaining 124 cases were of th t pe seen at any ENT doctor's consulting room

In all patients the hi tory of the disease was typical, with the complete triad f symptoms. A comprehensive audiological examination, including speech and Békésy audiometry was carried out in a large number of patients and pure tone audiometry was performed in all cases. The hearing tests were performed in attack free intervals.

The pure-tone audiograms were recorded with an *Amplivox* (Model 61) audiometer and the test were conducted under uniform conditions. Nar row-band maski g noise wa used. The intensity calibration was in ac cordance with the ISO standard (R 389 – 1964) The calibration was hecked regularly The hearing loss given in the figures represents the a erage of air conduction of 500 1000 and 2000 Hz.

Vestibular in estigation, including nystagmography with postural and cal ric tests, was performed in all patients. Medical, general neurological, ophthalmological and psychiatric examinations, EEG (Barac, Hagbarth & Stahle 1966) and X-ray of the temporal bone were performed as indicated.

RESULTS

Configuration of pure tone audiogram

The pure tone audiograms, which were recorded t the time of the first xamination, have been classified according to Carhart One modification has been introduced, however Curves classified by this author as gradual downward slope and marked downward sl pe" have been given the combined denotation of "f lling" curves in this study In our classification the stress has been placed on the general configuration of the pure-tone audiogram, and we ha e disregarded minor peaks and notches in an attempt t obt in n overall picture of the essential characteristics.

Curves of the flat type were most common (Fig. 1) and were found in

The flat curve is considered to be the most common, while rising and falling curves are regarded as more rare (Meurman & Grahne 1956 Meyer zum Gottesberge 1951 Lundborg 1960 Harbert & Young, 1963) Rossberg *et al* (1963) on the other hand claim that the trough shaped curve is the most usual and have designated this as pathognomonic of Menière s disease They have demonstrated this type of curve in 123 of 147 cases, and it is described as having first a downward slope followed by a rise to a peak at 1000–2000 Hz, and then at higher frequencies it either continues horizontally or falls at varying degrees Opheim & Flottorp (1957) on the other hand found in a smaller series of patients that the rising curve was the most common Goodman (1965) finally considers it impossible to distinguish typical groups, and has found that the most usual curve is a complex type characterized by sharp peaks and dips.

The high tone loss in the falling curve has been considered to be due to presbyacusis (Altmann 1955 Lindsay 1960) Meurman & Grahne (1956) have shown however that falling curves are not overrepresented in the higher age groups.

It is a general opinion that the hearing loss during the early stages of the disease affects mainly the lower frequencies, but later comprises the entire range (Meurman & Grahne, 1956 Walsh, 1958 Holmgren, 1964) Rossberg *et al* (1963) stated that the curve was usually trough shaped initially but became increasingly horizontal as the disease progressed Goodman (1965) finally claimed that there was no characteristic or systematic sequence of change of configuration of audiogram in the tem poral course"

The greatest hearing loss has been reported to occur within the frequency range below 3000 Hz (Opheim & Flottorp, 1957 Rossberg *et al* 1963 Goodman, 1965 and others)

Fluctuations of the auditory threshold are a characteristic feature of the disease pattern According to Meurman & Grahne (1956) and Schuknecht (1963) this phenomenon occurs chiefly during the earliest years of the disease most other authors, however have observed it, although less often in later stages (Lindsay 1949 1960 Simonton, 1956 Goodman, 1965 Opheim & Flottorp 1957) According to Goodman fluctuations of threshold occur irregularly at all frequencies, but Opheim & Flottorp have noted them mainly within the range 2000–3000 Hz.

Because of the many irregularly varying factors involved in the hearing loss, together with the greatly varying intervals between the vertiginous attacks, it seems to be difficult to distinguish any characteristic features of early or advanced cases.

The varying disease pattern which is so typical of this complaint has dominated the descriptions of Menière s disease The concept of the nature and course of the hearing damage appears to be rather vague We con sidered that an independent analysis of a large series of patients might contribute to some extent to increased knowledge of the character and

temporal development of the hearing loss. Since a large and uniformly investigated series had been collected at the Uppsala clinic over a period of a few years, such an analysis was found possible. The primary aim of our investigation was to study the configuration of the pure-tone audiogram in relation to the duration of the disease and to the degree of hearing loss. In addition, factors such as age at onset, onset symptoms, bilaterality and fluctuations are considered.

MATERIAL AND METHODS

The material consisted of 334 patients, of whom 160 (48%) were women and 174 (52%) men, and was collected consecutively over a period of just over 5 years. To a certain extent the material was selected, in that it included 210 patients who had such severe vertigo that they were treated surgically with ultrasonic irradiation. The remaining 124 cases were of the type seen at any ENT doctor's consulting room.

In all patients the history of the disease was typical, with the complete triad of symptoms. A comprehensive audiological examination, including speech and Békésy audiometry, was carried out in a large number of patients and pure-tone audiometry was performed in all cases. The hearing tests were performed in attack free intervals.

The pure-tone audiograms were recorded with an *Amplivox* (Model 61) audiometer and the tests were conducted under uniform conditions. Narrow-band masking noise was used. The intensity calibration was in accordance with the ISO standard (R 389 – 1964). The calibration was checked regularly. The hearing loss given in the figures represents the average of air conduction of 500, 1000 and 2000 Hz.

Vestibular investigation, including nystagmography with postural and caloric tests, was performed in all patients. Medical, general neurological, ophthalmological and psychiatric examinations, EEG (Barac, Hagbarth & Stable 1966) and X-ray of the temporal bone were performed as indicated.

RESULTS

Configuration of pure-tone audiogram

The pure-tone audiograms, which were recorded at the time of the first examination, have been classified according to Carhart. One modification has been introduced, however. Curves classified by this author as "gradual downward slope" and "marked downward slope" have been given the combined denotation of "falling" curves in this study. In our classification the stress has been placed on the general configuration of the pure-tone audiogram, and we have disregarded minor peaks and notches in an attempt to obtain an overall picture of the essential characteristics.

Curves of the flat type were most common (Fig. 1) and were found in

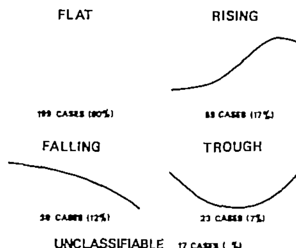


FIG 1 Configurati ns of pure-tone audiograms in 334 pati nts with Menière s dise se

199 patients (60%) The rising type was seen in 59 patients (17%) Usually we do not associate a falling type of audiogram with Menière s disease, but this type was recorded in 39 patients (12%) The average age of the patients with the falling type was 45 years, which may be compared with the average age of the total material which was 42 years. Marked trough shaped curves were noted in only 23 patients (7%) Finally the group with unclassifiable curves consisted mainly of patients with very severe hearing loss and fragmentary pure-tone audiogram curves.

The average hearing loss for the total material was 52 dB The hearing loss was, on the average less pronounced in patients with rising curves (44 dB) (Table 1) With flat and falling curves somewhat higher values were noted, viz. 50 dB and 54 dB respectively Among patients with un classifiable curves the hearing loss was very great, on the average 91 dB

The shortest average disease duration i e 5 years, was noted in patients with rising and trough types of audiogram while the average duration for patients with flat curves was 8 years (Table 1)

The influence of the duration of the disease on the magnitude of the hearing loss and also on the type of audiogram is shown in Table 2 It is evident that the hearing loss was least pronounced (35 dB) in patients with a disease duration of less than 6 months It then increased within a short period to an average of 50 dB, but after this time only small deteriorations in hearing were *observed with increasing duration of disease* The duration of the disease appeared to have very little effect on the percentage distribu tion of patients with different types of audiogram Calculated on the whole material flat curves were observed in 60% of the patients (Fig 1) and this figure was approximately the same with varying durations. One small difference was seen in the value for rising curves, which was somewhat higher (33%) in the group with the shortest duration of disease than the

TABLE 1 *Hearing loss and duration of disease related to type of audiogram in 334 patients*

Type of audiogram	No. of patient	Average hearing loss (dB)	Average duration of illness (years)
Flat	199	50	8
Rising	59	41	5
Falling	39	51	8
Trough	23	65	6
Unclassifiable	14	91	12

TABLE 2. *Hearing loss and types of pure-tone audiogram in 334 patients grouped by duration of disease*

Duration of illness (years)	No. of patients	Average hearing loss (dB)	Flat	Rising	Falling	Trough	Unclassifiable
			(The figures give the percentage of patients within the group)				
–½	43	35	53	33	7	2	5
½–1	19	50	67	11	11	11	—
1–2	25	50	64	16	8	8	7
2–3	30	49	47	30	13	10	—
3–4	37	52	65	19	13	3	—
4–6	21	64	43	14	5	21	14
6–	159	66	62	13	13	6	6

average for the whole material (17%). Trough curves were very seldom seen in the initial stage, but were observed more often after a duration of 6 months.

Fluctuation of auditory threshold

Repeated pure-tone audiograms were recorded in 161 patients, and the occurrence of fluctuations of threshold in this group was studied. Fluctuation is defined here as a recorded improvement of hearing with a change of hearing threshold of at least 10 dB at two test frequencies, or 15 dB at one test frequency. Flu tuations were recorded in a total of 76 patients (32%) (Fig. 2).

Flu tuations occurred in 34 out of 80 patients with flat curves. The greatest number of fluctuations were noted in patients with rising curves, but they were also common among patients with trough curves.

The fluctuation of threshold in relation to duration of the disease is seen in Fig. 3, which shows that this phenomenon was typical not only for recent cases, but also for patients with a duration of disease exceeding 5 years (6–20 years). *In general it may be said that approximately every econd patient exhibited fluctuating hearing regardless of the duration of*

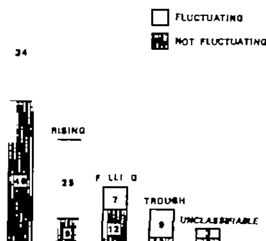


Fig. 2.

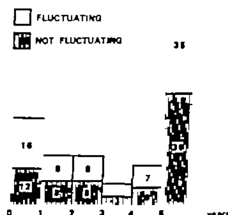


Fig. 3

Fig 2 Incidence of recorded fluctuation of he ring in 151 patient examined by repeated audi grams

Fig 3 Fluctuation f threshold in relation to duration of the disease in 151 pati ts.

the disease The position of the fluctuations on the frequency scale, and also their magnitude were studied in the 78 patients mentioned above and the results are presented in Fig 4 It is clearly evident from this figure that fluctuations of threshold were much more common in the range 250–1000 Hz than at higher frequencies

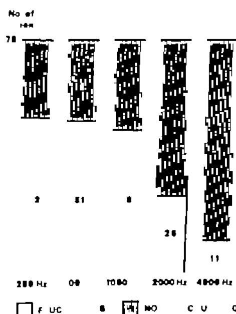


Fig. 4 Distributi n of threshold fluctu ti ns in relat t diff rent frequ nci f 78 patients with recorded fluct ati s. The figures i dicat the umbe f patients.

TABLE 3. *Average fluctuations in dB at different frequencies in relation to different types of audiogram in 78 patients*

Type of audiogram	Frequency 250 Hz	500 Hz	1000 Hz	2000 Hz	4000 Hz
Flat 24	25	25	26	26	21
Rising 25	29	34	31	23	32
Falling 7	18	28	25	25	0
Trough 8	21	22	28	21	0
Unclassifiable 14	23	23	23	28	40
Total	25	29	28	24	26

The average magnitude of the recorded fluctuations at different frequencies lay between 20 and 30 dB (Table 3). With flat and rising curves there appeared to be no evidence that the fluctuations were larger within any particular range of frequencies. With falling and trough curves, on the other hand, no fluctuations were noted at 4000 Hz. Because of the small number of patients in these two groups, however, no definite conclusions can be drawn in this respect.

Bilaterality

The material included 47 (14%) bilateral cases (23 men and 24 women). In 24 of these patients both ears were affected simultaneously or within the first year of illness. In the remaining 23 patients the second ear was involved after varying intervals of up to 20 years (Fig. 5). It should be stressed that in 17 patients (37%) there was a time interval of 5 years or more.

Among 287 unilateral cases 159 were left-sided and 128 right-sided, a relationship which is in agreement with previous reports (Cawthorne & Hewlett, 1954).

Age and onset of symptoms

The mean age for the whole material was 42 years, with no sex difference. The duration of the disease up to the time of the first examination was, on the average, 7 years. The ages of the patients at the time of the first symptom were: below 25 years, 26 patients (8%); 26–50 years, 230 patients (69%); 51–75 years, 77 patients (23%); over 75 years, 1 patient (0%).

Simultaneous hearing loss and vertigo was the most usual form of onset

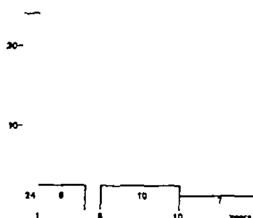


Fig. 5

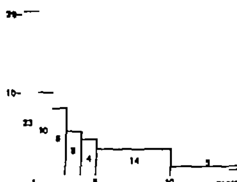


Fig. 6.

Fig 5 Elapsed time before involvement of the second ear in 47 patients with bilateral Menière's disease

Fig. 6 69 patients with hearing loss as first symptom Time elapse between first noted hearing loss and onset of vertigo

of the disease, being noted in 137 patients (41%) Vertigo alone as the first symptom occurred in 122 patients (37%) Least common as the first symptom was hearing loss alone, this being noted in 75 patients (22%) The *onset pattern in relation to the type of pure tone audiogram* showed no marked deviations from the general tendencies mentioned above

In 23 of the 75 patients in whom the first manifestation of the disease was hearing loss, vertigo occurred within one year In 41 patients the first vertigo attack occurred 1–10 years following the onset of the hearing loss, and in 5 patients intervals of up to 20 years were noted. For the remaining 6 patients no definite information was obtainable regarding the time of the first attack of vertigo (Fig 6)

TABLE 4 *Nature of onset in 334 patients classified by type of audiogram*

Type of audiogram	Hearing loss (No. of patients)	Vertigo (No. of patients)	Hearing loss and vertigo simultaneously (No. of patients)
Flat	43	75	81
Rising	11	21	21
Falling	8	10	15
Trough	9	4	10
Unclassifiable	4	3	7
Total	75 (22)	122 (37)	137 (41)

DISCUSSION

Two-thirds of the material upon which this investigation was based consisted of patients who because of severe vertigo had been referred to this hospital for surgical therapy (ultrasonic irradiation) Thus the majority of patients had an advanced form of Menière's disease and this must be taken into consideration when evaluating the results presented.

The great majority of pure-tone audiograms could be classified according to the method of Carhart Goodman (1965) pointed out the difficulties of distinguishing, in his material, pure-tone audiograms of the "classical type owing to the presence of deviations in the form of peaks and dips. In our classification into types we placed the chief stress on the general configurations of the pure-tone audiograms, and as a rule found no great difficulty in assigning them to their respective classes.

The distribution between the different types of pure-tone audiogram in a material of Menière patients is probably dependent on the nature of the material. Our material may be said to have consisted predominantly of severe cases, which may explain the high proportion of flat curves (60%) and the less common occurrence (17%) of rising curves. Trough curves were uncommon (7%) Any pronounced trough shaped curves should be evaluated with some caution, however since "false" trough curves can occur due to difficulties in masking the base frequencies satisfactorily in severe hearing loss.

The occurrence and significance of curves of the falling type in Menière's disease have been discussed in the literature and these have been regarded as a sign of simultaneous presbyacusi (Altmann, 19 5 Lindsay 1940) In our material, however the mean age for patients with falling curves differed ery little from that for the whole material. This observation supports the view of Björk (1964) and Meurman & Grahne (1956) that the high frequency loss in curves of the falling type is to be regarded as an xpression of Menière's disease.

The average duration of the disease was shortest in patients with rising and trough curves. With regard to curves of the rising type the observa tion that these were common in the early stage agrees with previous findings (Walsh, 1960 among others) As shown in Table 2, 33% of the patients with the shortest duration of disease showed this particular type of audiogram curve Rising curves also occurred, however in later stages of the disease This was evident by the fact that the average duration of the d sease in patients with thi type f urve was 5 years, and that in 12% of the patients with rising curves this duration exceeded 5 years. Similar results have been reported by Björk (1964)

Just as the ri ing curve is not limited to the early stage of the disease neither is the flat curve limited to the later stages. This was evident by the fact that half f ou patient with a disease duration of less than 6 months showed a flat curve. With regard to trough and falling curves, no definite

relationship was found with the duration of the disease Finally on comparison between the groups with flat rising and falling curves, it was noted that the average hearing loss was approximately of equal magnitude and lay between 44 and 54 dB This meant therefore that curves of the rising type also occurred in patients with advanced hearing loss

The average hearing loss reached a value of about 50 dB as early as one year after the onset of the disease, after which further deterioration, but of lesser degree slowly took place This prognostically unfavourable development may have been influenced to some extent by the composition of the material which included over 200 patients with such severe vertigo that operation (ultrasonic irradiation) was performed (Sjöberg & Stahle 1965) There appeared to be no direct parallelism however between cochlear and vestibular functional reduction the cochlea seems to be more vulnerable than the vestibular part of the inner ear This was evident in our previous studies on practically the same series of patients, where reduced caloric excitability was found in only 65% while hearing loss was noted in all patients *A considerable hearing loss during the early stages of the disease may therefore be expected as a characteristic feature of the general disease pattern*

It should be pointed out that the hearing loss has only been documented by the pure-tone audiograms, and that the discrimination capacity is not reported This should not alter the general trend, however Furthermore there appears to be no distinct relationship between the results of these two hearing tests since they are said to vary independently of one another (Cawthorne & Harvey 1953 Goodman 1965)

The early onset of hearing loss would seem to motivate greater therapeutic attention in the initial stage of the disease than has been perhaps generally paid hitherto *In spite of our deficient knowledge about the pathogenetic background of this complaint, every effort to prevent its advancement is indicated* We recommend commencement of consistent prolonged therapy *at an early stage* comprising intermittent administration of diuretics (Norell & Stahle 1961 1962 Stahle 1964 Klockhoff & Lindblom 1962, 1966) which can have a favourable effect on both hearing and vertigo

In order to determine the magnitude and nature of the auditory fluctuation continuous audiological control with frequent examinations are necessary Our investigation, like most previous studies, is retrospective and covers only the recorded fluctuations, while possible changes between examinations remain unknown No far reaching conclusions can therefore be drawn from the results obtained Three observations would seem to be relevant however for the disease as a whole (A) fluctuations occur in approximately every second patient (B) fluctuations occur with all types of curves, but somewhat more often with rising and trough curves than with the flat and falling types, and (C) fluctuations are on the whole equally common in the early as in the advanced stages of the disease

The occurrence of a flat curve cannot be interpreted as a sign that the hearing damage has become stationary and no longer has a tendency to regress. Among 80 patients with flat curves, fluctuation was recorded in 34 In agreement with Goodman (1965) we found that fluctuations occurred at all frequencies and were not associated with any particular frequency range However they were recorded more often at 250–1000 Hz than at frequencies above this range. The average magnitude of recorded fluctuations appears to be the same regardless of whether they occur in the higher or the lower range of frequencies, and in the present study they were of the order of 20–30 dB In the 7 patients with falling curves and fluctuations, no fluctuations were recorded at 4000 Hz. This may possibly indicate that the Menière's disease had not caused the high frequency loss.

As in studies of other authors, it was most usual for the disease to have its onset with hearing loss and vertigo simultaneously (41 %) Hearing loss alone as the first manifestation has been reported in the literature as the next most common form of onset, and vertigo alone as the least common (Day 1950 Cawthorne & Hewlett 1954 Rossberg *et al* 1965) Our results differ from these latter reports in that the disease was manifested with a vertigo attack in no fewer than 37 % of the patients, and with hearing loss alone in 22 % This distribution may have been due however t the composition of the material in which patients with severe vertigo were overrepresented.

On the basis of our own findings and those of other authors it may thus be said that in roughly *every second patient the onset of the disease is monosymptomatic* which may cause difficulties in differential diagnosis during the early stages. Acute severe vertigo with nystagmus and reduced caloric excitability may suggest vestibular neuritis (Stahle, 1966) Repeated ttacks of vertigo, sometimes apoplectiform without hearing loss, are de scribed as characteristic of the so-called Pseudo-Menière's syndrome. Simul t neous tinnitus together with a sensation of numbness in the ear indicate Menière's disease, however in both cases. In two-thirds of those patients in whom the hearing loss was the first symptom, more than one year elapsed before the first attack of vertigo (Fig. 6) An average time interval of 3 / years has been reported by Day (1950) Thus a cochlear hearing loss f initially unclear origin may be found with time to be caused by Menière's disease, although a considerable length of time may elapse before the onset of th characteristic vertigo.

Bilaterality was found in 14 % of our patients. Literature reports vary between 2.3 % and 28 % (Dandy 1941 Day 1946 Wright, 1948 Lindsay 1949 Castellano, 1951 Cawthorne & Hewlett, 1954 Altmann, 1955 Schuknecht, 1957 Barber 1964) The frequency of bilateral cases increases greatly however with increasing duration of the disease, which was shown in a previous study (Stahle & Bergman, 1967) based on the same material in a group f 102 patients, all with a disease duration of 10 years or longer

—

the frequency was no less than 25 %. The risk of involvement of the second ear is so great in longstanding cases, that this must be taken into consideration when making decisions with regard to surgery and the choice of operation method. It would seem that the number of total inner ear destructions should be reduced to a minimum in favour of methods aimed at the preservation of hearing. In approximately half of all bilateral cases in the present study involvement of the second ear occurred either simultaneously or within the first year of illness. In 17 out of 47 patients, however, there was an interval of 5 years or more. Our results differ in this respect from those of Day (1950), who found that in the great majority of bilateral cases both ears were affected simultaneously, and that in primarily unilateral cases subsequent involvement of the unaffected ear by the disease was so rare as to be negligible.

ACKNOWLEDGMENTS

The authors want to express their appreciation to Tore Lundborg, M. D., assistant professor, for reading and criticism of the manuscript and for valuable suggestions, and to Runo Jönsson, M. A., for invaluable help with statistical work and for skilful preparation of the diagrams.

ZUSAMMENFASSUNG

In 334 Fällen von Morbus Menière wurden Tonaudiogramme analysiert sowie Alter bei Erkrankung, Anfangssymptome und bilaterale Erscheinungen untersucht. Am häufigsten zeigte das Tonaudiogramm einen horizontalen („flachen") Verlauf (60%), gefolgt von einer steigenden Kurve (17%) und einer fallenden Kurve (12%). Der durchschnittliche Hörverlust für das gesamte Material betrug 52 dB. Patienten mit horizontalen, steigenden und fallenden Tonaudiogrammkurven hatten einen Hörverlust von etwa derselben Grössenordnung. Patienten mit steigenden und wannenförmigen Kurven hatten die durchschnittlich kürzeste Krankenzeit. Der Hörverlust schritt am schnellsten während des ersten Jahres nach der Erkrankung fort, danach war die Zunahme geringer. Das Durchschnittsalter für Patienten mit fallenden Tonaudiogrammkurven war etwa dasselbe wie für das gesamte Material.

Schwankungen der Hörschwelle wurden bei sämtlichen Kurventypen festgestellt, waren aber etwas häufiger bei steigenden und wannenförmigen Kurven. Schwankungen wurden in etwa der Hälfte aller Fälle festgestellt, und zwar unabhängig von der Dauer der Krankheit und häufiger im Bereich 250–1000 Hz als bei höheren Frequenzen. Die Grösse der gemessenen Schwankungen betrug im Durchschnitt 20–30 dB und war beinahe unabhängig vom Frequenzbereich.

Bei zweiseitigen Fällen (14%) wurde das andere Ohr bei der Hälfte gleichzeitig oder während des ersten Jahres nach der Erkrankung befallen. Bei den übrigen Fällen erkrankte das andere Ohr nach einer Zeit bis zu 20 Jahren. 77 der Patienten erkrankten vor dem 51. Lebensjahr. Gleichzeitiges Auftreten von Hörverlust und Schwindel war die häufigste Kombination beim Eintreten der Krankheit (41%). Schwindel als Anfangssymptom wurde bei 37 und Hörverlust bei 22% der Fälle festgestellt. Bei zwei Dritteln der letztgenannten Fälle trat der erste Schwindelanfall erst nach ein oder mehreren Jahren auf.

REFERENCES

Altmann, F. 1955. Morbus Menière. *Fortsch. Hal. Nas.-Ohrenh.* Ilk. 2. 1. 5. Karger Basel and New York.

Barac, B., Hagmantr, K. E., and Samuel, J. 1966. EEG in Menière disease A tudy f EEG and caloric directional preponderance before and after ultrasonic irradiation of the labyrinth. *Act. Otolaryng.* (Stockh.) 62 332.

Barber, H., 1964. Positional nystagmus especially after head injury. *Laryngoscope* 74 891.

Bolas, H. 1964. The prognosis i. Morbus Menière. *Acta Otolaryng.* (Stockh.) Suppl. 188, 137.

Carhart, C. R., 1945. An improved method for l. lfyi g audiograms. *Laryngoscope* 55, 640.

Castellano, R., 1951. Menière disease and its surgical treatme t. Report of 300 cases. *J. Y. Surgery* 1, 172.

Cawthorne, T. E., and Hanves, R. M. 1953. A comparison between heari g for pure tones and for speech. *J. Laryng.* 67 232.

Cawthorne, T. E., and Hewlett, A. B., 1954. Menière' disease. *Proc. Roy. Soc. Med.* 47 663.

Davis, W. E., 1941. The surgical treatment of Menière disease. *Surg. Gynec. Obstet.* 72, 421.

D K. M., 1948. Hydrops of labyrinth (Menière disease). Diagnosis—Results of labyrinth surgery. *Laryngoscope* 58 33.

— 1950. Menière disease. Present concept of diagnosis and management. *Ann. Otol.*, 59 964.

Goodman, A. C., 1965. New observ ti ns on changes in hearl g i the temporal course of Menière disease. *Ann. Otol.* 74 991.

Harvent, F. and You. g, I. M., 1963. Audiologi findings in Menière syndrome. *A t. Otolaryng.* (Stockh.) 57 145.

Holmgren, L., 1961. Hearing tests i Menière disease. *Act. Otolaryng.* (Stockh.) Suppl. 192 118.

Klockhoff, I. and Lindblom, U. 1962. Behandling syndroma Menière med hydrochlortiazid. *Nord. Med.* 67 789.

— 1966. Endolymphatic h drops revealed by glycerol test. *Act. Otolaryng.* (Stockh.) 61 459.

Lindsay, J. R., 1949. Labyrinthine surgery for Menière disease. *Laryngoscope* 59 22.

— 1960. Hydrops of the labyrinth. *Arch. Otolaryng.* (Chic.) 71 500.

Lu pnoma, T. 1960. Some experiences with the Bekesy-audiogram i end organ and nerv fibre deafness. *Int. Soc. Audiol. Compt. R. d. V Congr.* 227.

Mer s, O. H., and Grahne, B. 1956. Heari g i Menière' disease. *Pract. Otorhinolaryng.* (Basel) 18 283.

Meyer Gottfriedrich, A. 1951. De Höchbild der gemeine Menièreschen Krankheit. *Ar h. Oh. Nas.-Kehlk. pfheilk.*, 159 482.

Norell, L. and Samuel, J. 1961. Hydroklortiazid vid morbus Menière. *N rd. M d.* 66 1637.

— 1962. Treatment of Menière disease with hydrochlorothiazid. *Act. Otolaryng.* (Stockh.) 54 447.

Ortmann, O., and Flottorp, O., 1957. Menière's disease. Some audiological and cli ical observ tions. *Acta Otolaryng.* (Stockh.) 47 292.

Persson, L., 1953. Syndroma Men ère och dess behandling. *Nord. Med.* 49 201.

Rosenthal, G., Schwartz, H., Hevyer, K., and Beck, L. K. L., 1963. Zu Diagnose, Therapie

und Prognose der Menièreschen Krankheit (Erfahrungsbericht der Klinik) *Z Laryng Rhinol Otol* 5 316

SCHUKNECHT H F 1957: Ablation therapy in the management of Menière's disease *Acta Otolaryng* (Stockh.) Suppl *132* 1

— 1963 Menière's disease A correlation of symptomatology and pathology *Laryngoscope* 73 651

SIMONTON K M, 1956: End-organ deafness. Diagnosis and significance *Arch. Otolaryng* (Chic.) *63* 262

SJÖBERG, A and STAHLE, J., 1965 Treatment of Menière's di ease with ultrasound *Arch Ot laryng* (Chic.) *8*? 498

ST HLE, J 1964 Hydrochlorothiazide in the treatment of M ière disease *Act Otolaryng* (Stockh.) Suppl *188* 209

— 1966 Vestibular neuritis The vestibular system nd its diseases. *Trans Int Vestibula Sympos* Grad school medic., Univ Pennsylvania, U iversity of Pennsylvania Press, Philadelphia

STAHLE, J and BERGMAN, B 1967: Th caloric reaction in Menière's disease *Laryngoscope* 77 1629

WALSH, T E., 1958 Menière's disease *Laryngoscope* *68* 279

WRIGHT A J 1948 Menière's disease. *Proc Roy Soc Med* *41* 801

Dept of Otolaryngology University Hospital
Uppsala 14 Sweden

Received May 10 1967

CALCIOPEN®-K

tabletter à 600 000 IE

Jämforande antibakteriella aktivitetsbestamningar har bekraftat de kliniska iakttagelserna att V penicillin fortfarande forsvarar sin plats som forstahandspreparat vid penicillinbehandling For att sakerställa terapisvar även vid infektioner fororsakade av stammar med något mindre kanslighet for penicillin har man mer och mer overgått till hogre dosering

CALCIOPEN® K tabletten innehåller 600 000 IE Den är spolformad filmdragerad och lätt att svälja

DEKLARATION

Varje tablett innehåller
Phenoximethylpenicillinkalium 0,4 g
(respond 600 000 IE)

INDIKATIONER

Alla infektionssjukdomar förorsakade av penicillinkänsliga bakterier

KONTRAINDIKATION

Penicillinöverkänslighet

DOSERING

Vuxna 4 tabletter per dag (1 på morgonen 1 vid middagstiden och 2 till natten)

FÖRPACKNINGAR

Glas à 20 30 40 100 och 250 tabletter

Sjukhusforpackningar à 10×100 tabletter och 5×250 tabletter

LEO • HÄLSINGBORG

INFLUENZA CAUSES GREAT LOSSES TO SOCIETY

The epidemic of influenza in 1957–1958 was calculated to have reduced the total volume of work during a year in Sweden by 0.29%—a loss of 5.5 million work-days, corresponding to about 9 million pounds. (1)

FLUMIDIN prophylaxis gives 40–94% protective effect during an influenza epidemic with high frequency of morbidity (2)

FLUMIDIN®



Results of clinical investigations (2)

Type of virus infection	*Medication*	*Dosage schedule*	*Reported result*
Influenza			
High morbidity (10–18%)	Prophylaxis	100 mg t.i.d.	39% protection
	Prophylaxis	200 mg t.i.d.	From 40% to 94% protection
Low morbidity (-4%)	Prophylaxis	200 mg t.i.d.	From no observable effect to 78% protection
Upper respiratory infection	Prophylaxis	100 mg t.i.d.	From no observable effect to 51% protection
	Prophylaxis	200 mg t.i.d.	From 42% to 67% protection
	Prophylaxis	400 mg b.i.d.	From statistically not significant to 70% protection
Morbilli (measles)	Prophylaxis	500 mg t.i.d.	No observable effect
Varicellae (chicken pox)	Prophylaxis	500 mg t.i.d.	No observable effect
Influenza	Therapy (suppression)	200–500 mg t.i.d.	Statistically significant reduction of average illness period by bout one third; from > 6 days to about 4 days
Upper respiratory infection	Therapy (suppression)	200 mg t.i.d. 400 mg b.i.d., t.i.d.	From no observable effect to statistically significant reduction of number of cases with illness period above 5 days
Herpes zoster	Therapy (suppression)	200 mg t.i.d. 400 mg b.i.d.	Rapid relief of pain and mitigation of neurological manifestations, inhibition of propagations
Morbilli (measles)	Therapy (suppression)	500 mg t.i.d.	No observable effect
Parotitis (mumps)	Therapy (suppression)	500 mg t.i.d.	No observable effect
Polio	Therapy (suppression)	5 mg b.i.d.	No observable effect
Varicellae (chicken pox)	Therapy (suppression)	500 mg t.i.d.	No observable effect

Flumidin® 400 mg tablets à N,N [Anhydrobis (2 hydroxyaethyl)] biguanide hydrochlor (ABOB)

Indication Influenza (prophylaxis)

Dosage Adults and children over 5 years of age: 400 mg twice daily (morning and evening). Children under 5 years of age: 200 mg three times daily

Packs 20, 100 and 1000 tablets.

(1) Kördof, B 1957–1958 års influensaepidemi i Sverige. Sv Läkartidn. 27 (1959) p. 1911

(2) Flumidin — samlade erfarenheter med ABOB AB Kabi, Stockholm 1965 p 8 (90 pages, partially in English, available upon request from AB Kabi Stockholm 30).

Detailed information is available on request from AB KABI Stockholm 30 Sweden

AB KABI STOCKHOLM 30 SWEDEN